TMA Module for MIRAX Viewer 1.12

User's Guide

June 11, 2009

3DHISTECH

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Font Types and Symbols \ Keyboard Quick Reference

Font Types and Symbols

Example	Words or characters that appear on the screen. These include field names, screen titles, pushbuttons and menu names, paths or options. Keys on the keyboard. For example, function keys (such as **F11**) or the **Ctrl+O** key combination.
Example	Cross-references to other documents.
Warning!	Indicates that you need an additional module to use the feature or that there are prerequisites for the task.
Important!	Contains an important piece of information or a recommendation. The application will work if you choose not to follow the recommendation but its performance might be less than optimal.
Tip!	Contains a suggestion about using the application in some other way or to some interesting purpose.

1 About the TMA Module for MIRAX Viewer 1.12

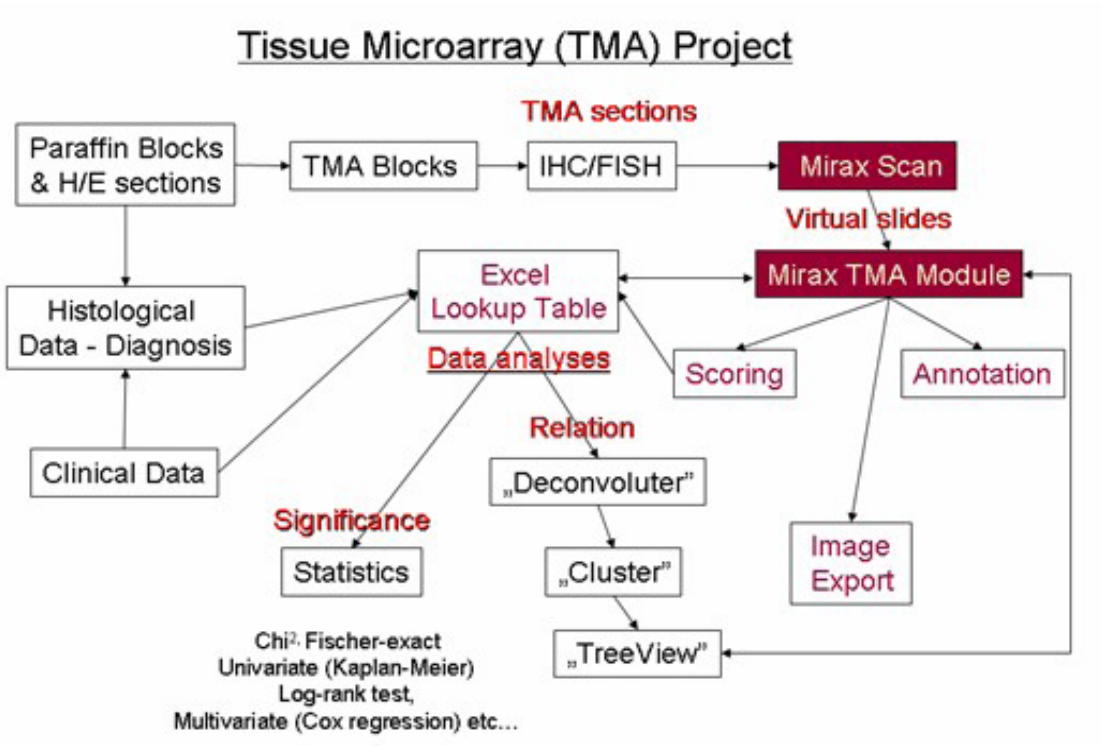
The TMA Module for MIRAX Viewer allows you to perform molecular morphological analyzes on slides digitized with MIRAX scanner microscopes. With this module, you can digitally inspect and score stained tissue micro array (TMA) slides on your computer screen. After you scored the slides, you can link the image files with pathological and clinical data in a shared database and further submit them to statistical, cluster or survival analyses.

Important!

Slides digitized with other scanner microscopes have not been tested with MIRAX TMA module. Therefore, the features described in this document are fully supported only for slides that have been digitized with MIRAX SCAN, MIRAX SCAN2, MIRAX MIDI or MIRAX DESK.

1.1 Overview of the TMA Module for MIRAX Viewer 1.12

The role of MIRAX TMA Module software in the workflow of a TMA project is shown in figure below (IHC = immunohistochemistry; FISH = fluorescence in situ hybridization)



1.2 New Features of the TMA Module for MIRAX Viewer 1.12

- Spot finding:
  - Batch mode.
  - Storing profiles (if using several slides, the software saves your previous settings).
  - Grid retorsion according to rows and columns.
  - Grid retorsion according to spots.
  - Manual anchoring for spots.
  - Activating grid.
  - Deleting grids (the last grid will be active).
  - Reverse spot numbering.
- Adding new slide to an existing project.
- Image export without database import.

2 Installing TMA with MIRAX Viewer 1.12

TMA is not a standalone application: you need MIRAX Viewer to run it. Therefore, you have to install MIRAX Viewer before installing the application.

2.1 Prerequisites

Tools to be Used	Description of Prerequisites
MIRAX Viewer version 1.12 TMA Module \ Excel Export	Software: Runs on Microsoft Windows **XP** and **Vista 32 Business** Microsoft **Office** or Open Office
	Hardware: Recommended: **Pentium C2D (DualCore), 2 GHz, 2GB RAM** Minimum: Pentium P4, 3GHz, 1GB RAM Minimum Disk Space: 110 MB Mouse with scroll wheel

2.2 Installing the Application

1. Start the **MIRAX_Viewer.exe** file that you received on a DVD or downloaded in a compressed file.

2. Follow the instructions in the Installation Wizard.

3. Start the **3DHISTECH_Apps.exe** file that you have purchased. Make sure, that you also have the dongle or the **MView.lic** licence file.

4. Follow the instructions in the Installation Wizard. When prompted, select the dongle or browse for the licence file. Choose the application TMA during installation.

3 Setting up a TMA Project

3.1 Collecting Project Data in an Excel Worksheet and Building a TMA

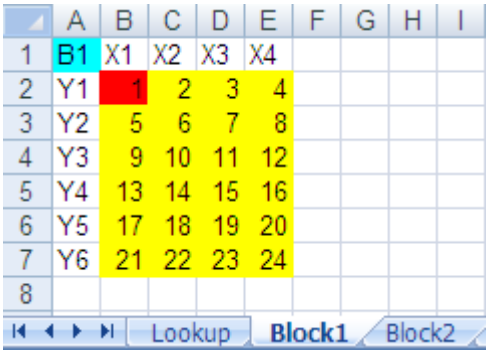
A TMA project can be started with collecting pathological and clinical data of relevant patients in Excel data sheet format (TMA Lookup table) and using the individual donor blocks correlated with their H&E stained sections for punching out representative cores and producing TMA blocks either using an automated or a manual TMA arrayer. TMA Master instrument is available to support MIRAX TMA projects.

Each Excel file supporting a project (TMA database file) should include at least three worksheets: One for the main Lookup table (Lookup); one (or more) for linking stained spots with their location in the TMA blocks (Block1..Blockn); and one for linking the stains to Excel Lookup table data (Slides).

	A	B	C	D	E	F	G	H	I	J	K
1	Unique TMA Sample ID	TMA Block ID	Donor Block ID	Organ	Diagnosis	Grade	Sender institute	Survival	Stage	Metastasis	X
2	1	Block1	1722-02	liver	cirrhosis	G0	Szombathely				
3	2	Block1	546-04	breast	IDC	G2	Bay				
4	3	Block1	546-04	breast	IDC	G2	Bay				
5	4	Block1	210-04	breast	Mucinosus carcinoma	G2	Bay				
6	5	Block1	210-05	breast	IDC	G3	Bay				
7	6	Block1	1643-04	breast	IDC	G3	Bay				
8	7	Block1	1416-04	breast	ILC	G2	Bay				
9	8	Block1	1610-04	breast	IDC, comedo	G2	Bay				
10	9	Block1	128-05	breast	ADC metastasis	poor	Bay				
11	10	Block1	774-02	breast	connective tissue	G0	S.E. I. Pathology				
12	11	Block1	659-02	breast	ILC	G3	S.E. I. Pathology				
13	12	Block1	322-02	breast	IDC	G2	S.E. I. Pathology				
14	13	Block1	1315-02	breast	IDC	G2	S.E. I. Pathology				
15	14	Block1	1199-02	breast	IDC	G3	S.E. I. Pathology				
16	15	Block1	4761-02	breast	DCIS	G0	S.E. I. Pathology				
17	16	Block1	657-03	breast	connective tissue	G0	Bay				
18	17	Block1	1222-03	breast	IDC	G2	Bay				
19	18	Block1	1324-03	breast	ILC	G1	Bay				
20	19	Block1	1643-04	breast	IDC	G2	Bay				
21	20	Block1	1643-04	breast	IDC	G2	Bay				
22	21	Block1	1826-02	breast	DCIS	G0	S.E. I. Pathology				
23	22	Block1	1877-02	breast	ILC	G2	Natl. Inst. Cancer				
24	23	Block1	988-03	breast	IDC	G2	Bay				
25	24	Block1	1023-03	breast	IDC	G0	Bay				
26	25	Block2	210-05	breast	connective tissue	G3	Bay				
27	26	Block2	1643-04	breast	ILC	G2	Bay				
28	27	Block2	1416-04	breast	IDC	G2	S.E. I. Pathology				
29	28	Block2	1610-04	breast	IDC	G3	S.E. I. Pathology				

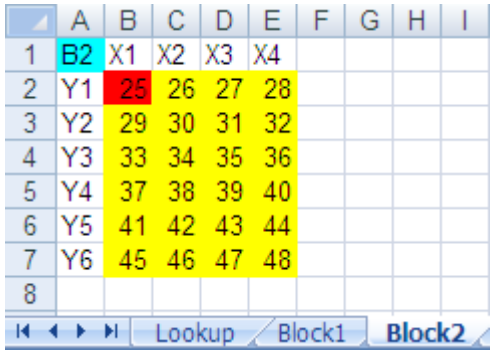
Lookup | Block1 | Block2 | Slides

For the sake of safe tissue core (stained spot) identification (see later at Spot Finding), it is essential to include accurate information into columns A and B and C of the Lookup table. All other columns are required for the final correlation analyses with staining profiles.



	A	B	C	D	E	F	G	H	I
1	B1	X1	X2	X3	X4				
2	Y1	1	2	3	4				
3	Y2	5	6	7	8				
4	Y3	9	10	11	12				
5	Y4	13	14	15	16				
6	Y5	17	18	19	20				
7	Y6	21	22	23	24				
8									

Lookup Block1 Block2



	A	B	C	D	E	F	G	H	I
1	B2	X1	X2	X3	X4				
2	Y1	25	26	27	28				
3	Y2	29	30	31	32				
4	Y3	33	34	35	36				
5	Y4	37	38	39	40				
6	Y5	41	42	43	44				
7	Y6	45	46	47	48				
8									

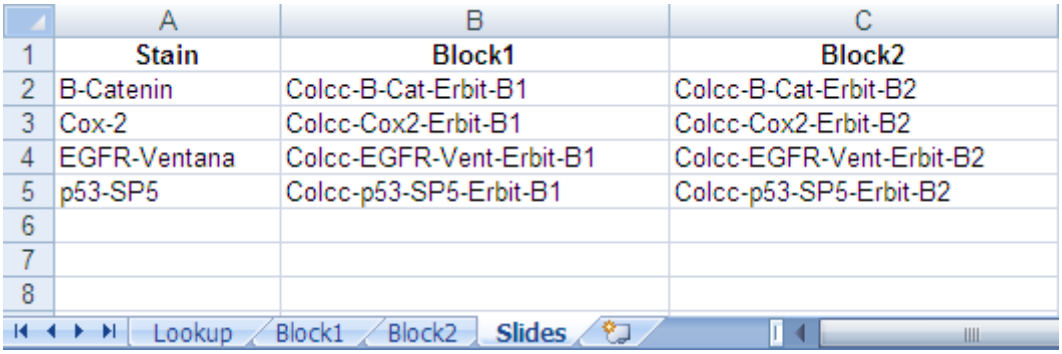
Lookup Block1 Block2

Either using a manual or automated arrayer, a TMA map should be designed for each block to show the location and orientation of tissue cores according to the TMA block ID (Block1, Block2, Block3 etc…). This map will be used later at Spot Finding. The serial numbers in the TMA map correspond to the Unique TMA sample ID of the Excel data sheet. If there are clusters of cores within a TMA block they should also be numbered on the same basis including all clusters of a block into the same worksheet as they are arranged in the block.

Tip!

The core behind the red label in the corner of each TMA block map is an orientation tissue easily differentiated from the test tissue type (for example liver). However, one can use any other ways for easily recognizing the original orientation of the TMA section. Leaving out cores may be an option, but please consider that the depth of the cores is different and some may be used up at cutting before others, which also result in missing cores.

The third worksheet in the Excel database file identifies stains used in the particular project. In the Stain column one can indicate the official, may be full name of the detected antigens, for example with the given clone used. In the block column (that is, Block1 for TMA Block 1, Block2, Block3… for further blocks in the project), one must use the very same labels which had been used for labeling the given slide at scanning. In other words, a slide in each TMA block column is best defined by the serial number of the slide, the applied marker and the TMA block ID (that is, the slide named 12-E-Cadh is the 12th section of Block2 block stained for E-Cadherin marker).



	A	B	C
1	**Stain**	**Block1**	**Block2**
2	B-Catenin	Colcc-B-Cat-Erbit-B1	Colcc-B-Cat-Erbit-B2
3	Cox-2	Colcc-Cox2-Erbit-B1	Colcc-Cox2-Erbit-B2
4	EGFR-Ventana	Colcc-EGFR-Vent-Erbit-B1	Colcc-EGFR-Vent-Erbit-B2
5	p53-SP5	Colcc-p53-SP5-Erbit-B1	Colcc-p53-SP5-Erbit-B2
6			
7			
8			

Lookup | Block1 | Block2 | Slides

For further instructions on slide scanning please refer to the User Guide of MIRAX Scan.

Important!

For identifying the position of consecutive sections in Z-dimension /depth/, it is essential to use serial numbers in front of the slide labels when scanning with the MIRAX Scan (see in column Block1).

It is also useful to select the same order of sections for immuno-staining different markers in different blocks (for example E-Cadherin is followed by EGFR, etc… in each block).

However, these features are optional and slides that are not labeled in this way may also be supported by the software.

When starting a project, all information included in the Excel database file can be imported into the MIRAX TMA software, to be exported into a new Excel file added with the scores when scoring will have been finished.

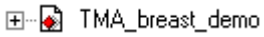
3.2 Creating a New TMA Project

3.2.1 Before You Start

Make sure that the slides that you want to add to the TMA project are all in one folder at a location that you can access from MIRAX Viewer and that they are not 'read-only'.

3.2.2 Creating a New TMA Project

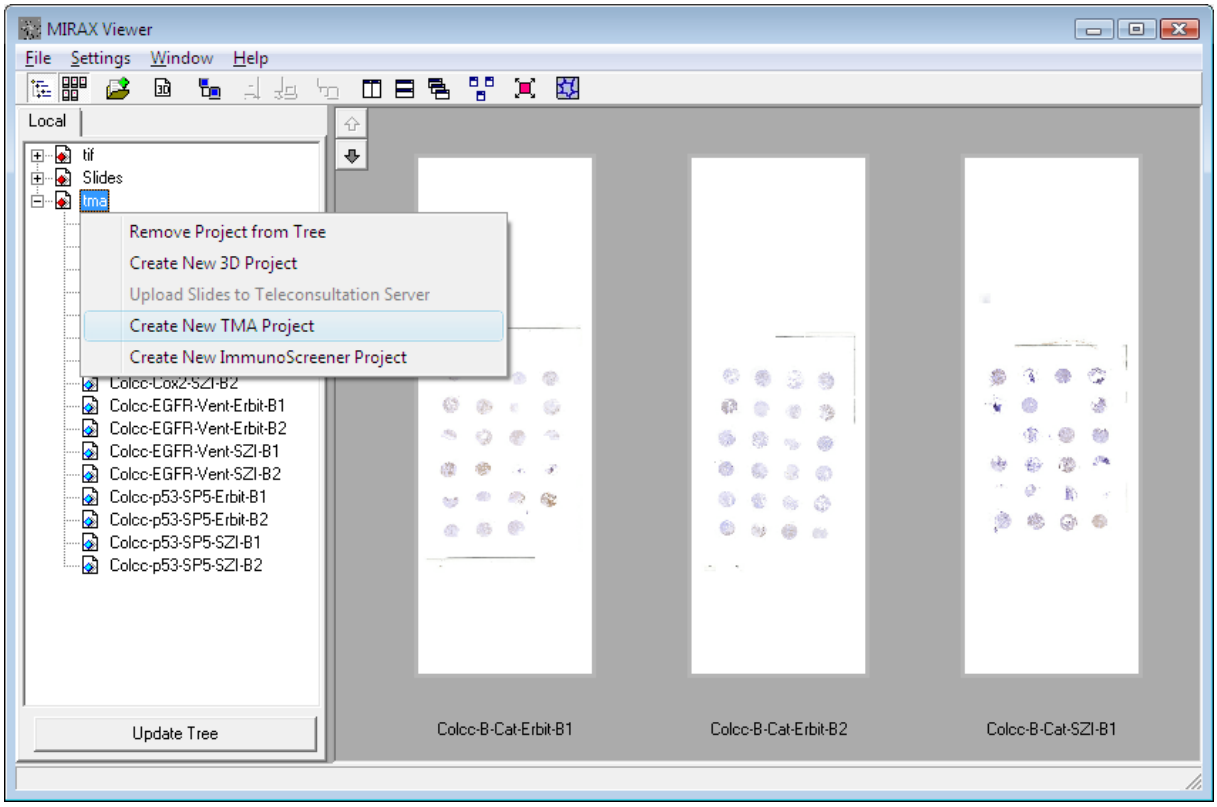
For creating a new TMA project, click on the Slide-project folder in the Slide Tree panel.



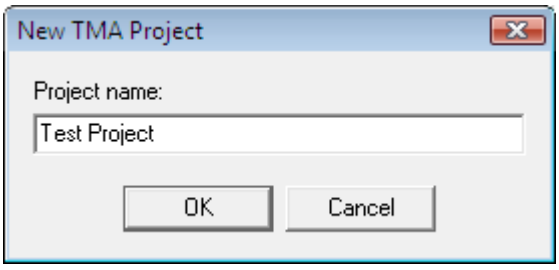
Clicking on the "+" sign will list all slides found in the given folder, while in the right side panel their preview image will be opened with the same labels at the bottom, which were used at their scanning.

1. Click on the Slide Project folder with the right mouse button.

2. Select **Create New TMA Project** from the menu. Then the new project will include all digital slides found in the original Slide project folder



3. Enter a name for the new project in the **Project name** text box, then click **OK**. As it cannot be modified later, be sure to incorporate all the necessary information into the name of the new project. The **TMA Project** will be located within the folder of its slides, which can be visualized after pressing **Update Tree** in the bottom left part of the Mirax Viewer screen



4. The **TMA Project Wizard** dialog appears.

5. User Management dialog:

   Since the software offers parallel scoring for several users, enter a user name in the **User Name** text box, then click **Next**. The user will score the slides. If you need a multi-user project, you can add further users later on, when the project has been created and you first start the project.

6. Slide Selection:

   a. Select the appropriate slides for all the TMA slides.

      - The **Path** text box displays the path for the TMA slides.
      - To select all slides, click .
      - To deselect all staining types, click . The slides that you leave out are not removed from the file system. You can also add them or new ones to the project later.
      - To display the thumbnails for the TMA slides, click .
      - To display the TMA slides in list view, click .

   b. After selecting the appropriate slides, click **Next**.

7. Project Data:

   To import the Excel database (ID, specimen, diagnosis, etc.), click **Find DataBase** and browse for the appropriate Excel file. When finished, click **Next**.

8. Spot Finding:

   a. To ignore spot finding, select **Ignore Spot Finding**. You can access the spot finding dialog later, by clicking **Spot Finding** in the TMA Project window.. For more information on Spot Finding, see section ***3.7 Finding TMA Spots***.

   b. For manual spot finding, select **Manual Spot Finding**. This will open the **Spot Finding** dialog instantly after finishing the steps of the wizard.

   c. Click **Finish**.

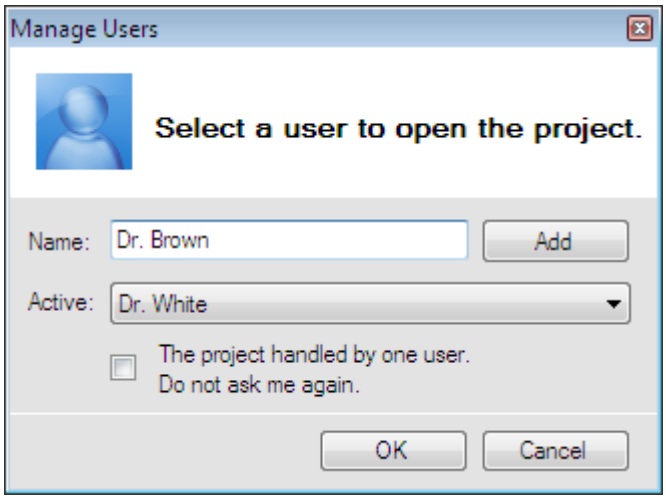
3.3 Opening a Project

Existing projects are marked with a TMA icon, followed by the name of the project. Double clicking on the given project icon will open the project folder. The software will ask for the scorer, if it is only the same person who created the project, go ahead. If different, **Add** new user to the list. In case of more than one assessors in a project, the Consolidate option is automatically added, which can either be used as a separate scorer to decide the final consensus scores, or this function will offer automated creation of consensus scores based for example on mean values of different scores.

If the project is a one-user project, select the **"The project handled by one user"** check box to disable the notification.

Important!

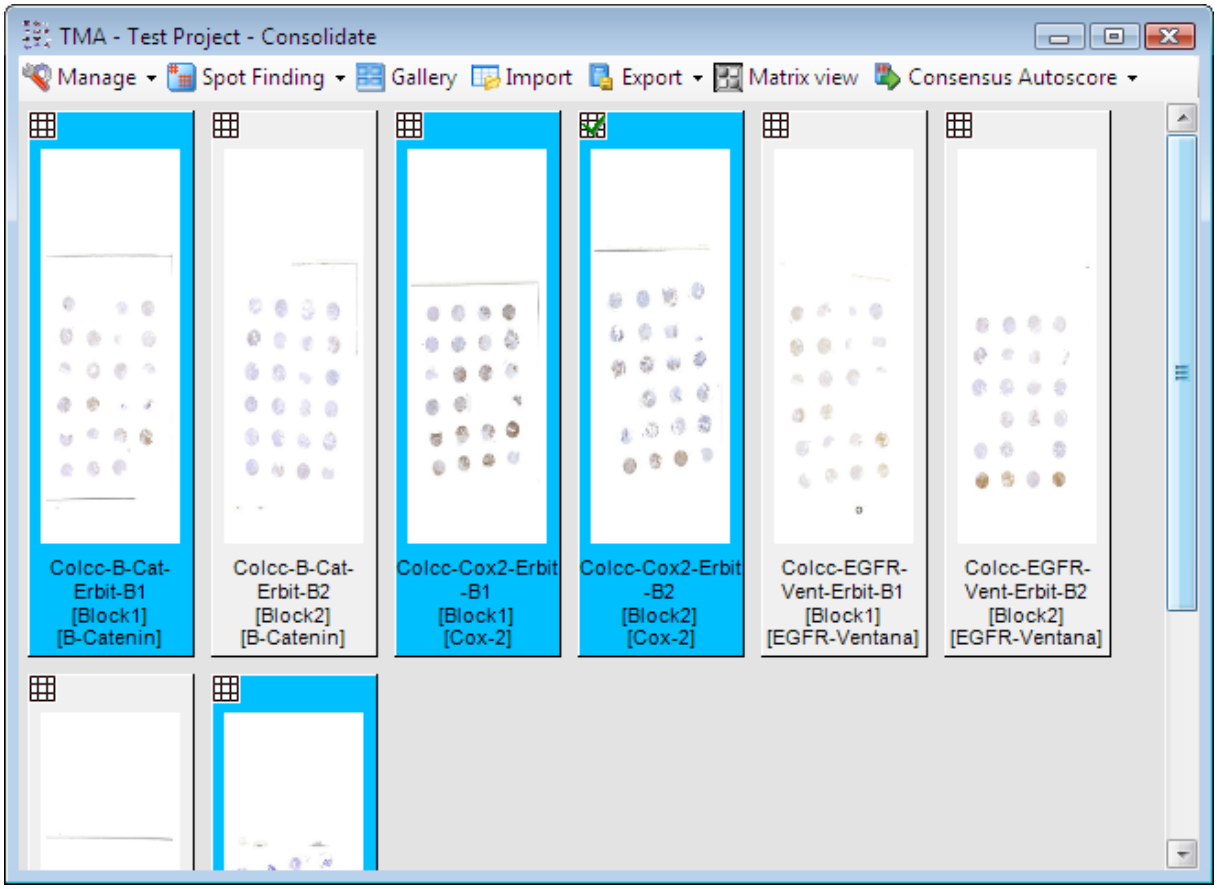
If you have selected the **"The project handled by one user"** option, you will not be able to add more users to the project later.



To remove obsolete slides from a project click on the annotation of a slide with the right mouse button and select **Remove Slide(s)**.

3.4 Slide selection

To select more than one slide for removal, keep the **Ctrl** button pressed and click on the slides to be removed. These slides will be removed from the project but will not be deleted from the disk.

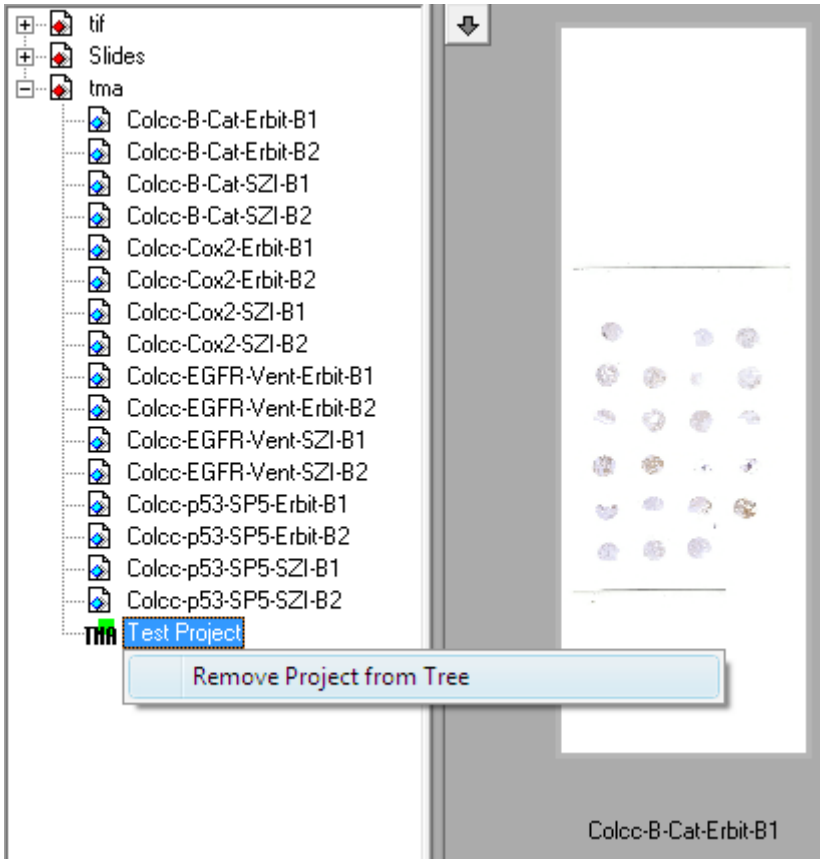


Important!

To avoid confusion only one project is allowed open at a time.

3.5 Remove Project

Existing TMA projects can also be removed by clicking on the project name with the right mouse button and choosing Remove project from Tree.



3.6 Managing TMA Projects

Having started a new TMA project or opened up an existing project the function buttons available for project management are listed on the top of the panel.

Important!

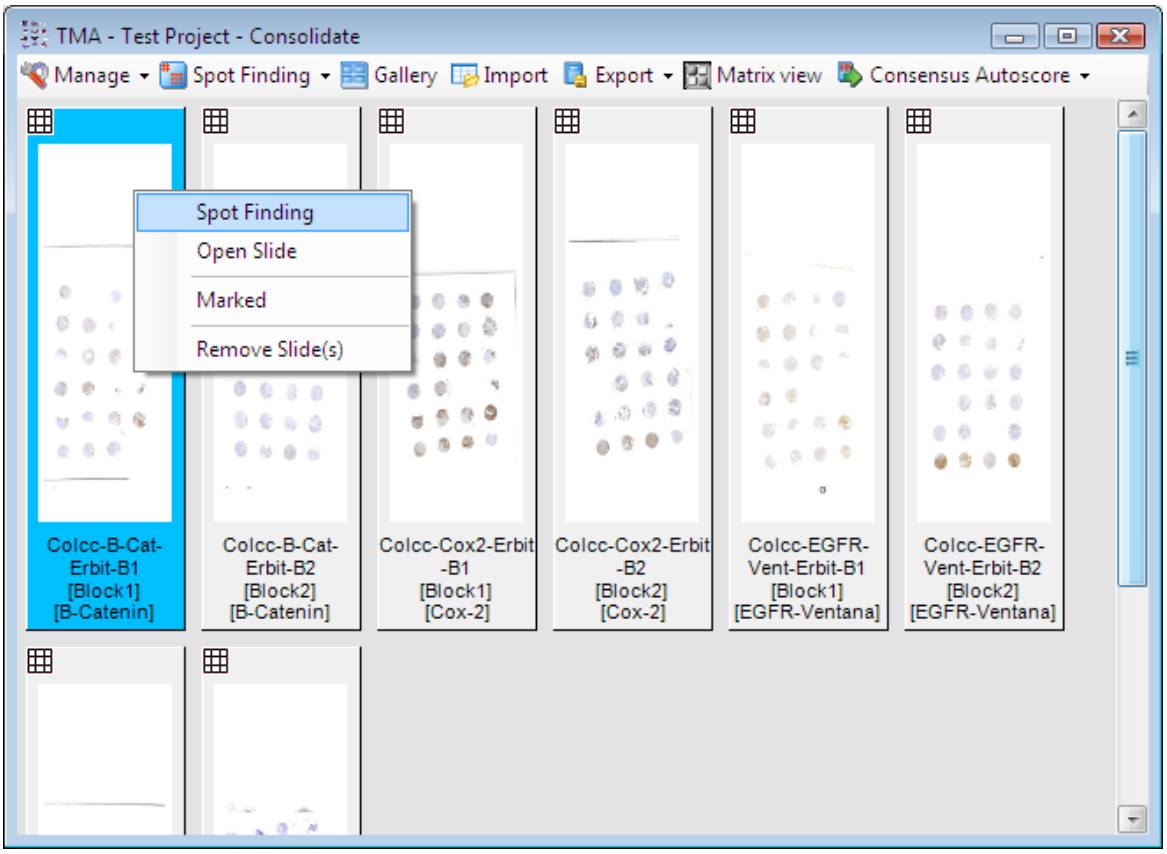
You can only see all function buttons if it is a one-user project, or if you are signed in as Consolidate user in a multi-user project. If you are signed in as another user in a multi-user project, you cannot access all functions.

- Gallery – Allows for creating TMA spot galleries according to any filter options represented by each column in the Excel Lookup file.

- – Allows importing database from Excel in XLS format.
- – Allows exporting database to a new Excel file merging original Lookup table data with the newly generated scoring data.
- – Allows opening the selected slides, adding or removing slides and creating either simple or combined schemes. (Look at the details in chapter 4.1) Always put new slides in the folder where all the other slides in the project are.
- – This button appears only for Consolidate user to make possible the comparison of the of the ordinary users' scores.
- – Sorts slides in the following order: Each row represents one TMA block displaying consecutive sections (Z-dimension /depth/). Each column lists sections of consecutive blocks stained for the same marker below one another.

3.7 Managing TMA Slides in a Project

Having started a project, there are four functions to select from the pop-up menu when clicking on a slide with the right mouse button.



- The Spot Finding option allows for identifying and linking to Excel data of each stained spots within the given TMA slide (block).

- The Open Slide option allows for opening a slide in the MIRAX Viewer software.

- By selecting the Marked option you can indicate with a green tick label in the grid of a slides annotation that the scoring of that slide is completed. (Please note that this option will not be activated automatically when scoring has finished!)

- The Remove Slide option is used for eliminating the given slide from a project (see above). To remove obsolete slides from a project click on the annotation of a slide with the right mouse button and select **Remove Slide(s)**. These slides will be removed from the project but will not be deleted from the disk.

Important!

You can only remove slides from a project if it is a one-user project, or if you are signed in as Consolidate user in a multi-user project. If you are signed in as another user in a multi-user project, you cannot access this function.

The slide label including the serial section number (Z-dimension /depth/), stain label and block number appear in the bottom region of the thumbnail image of each slide. A grid label seen in the upper left corner of each slides annotation indicates that the spots within the given slide have been identified by using the Spot Finding (The green tick label for Marked appears if activated, and may refer to the completed scoring).

3.8 Linking Excel Data to TMA Spots

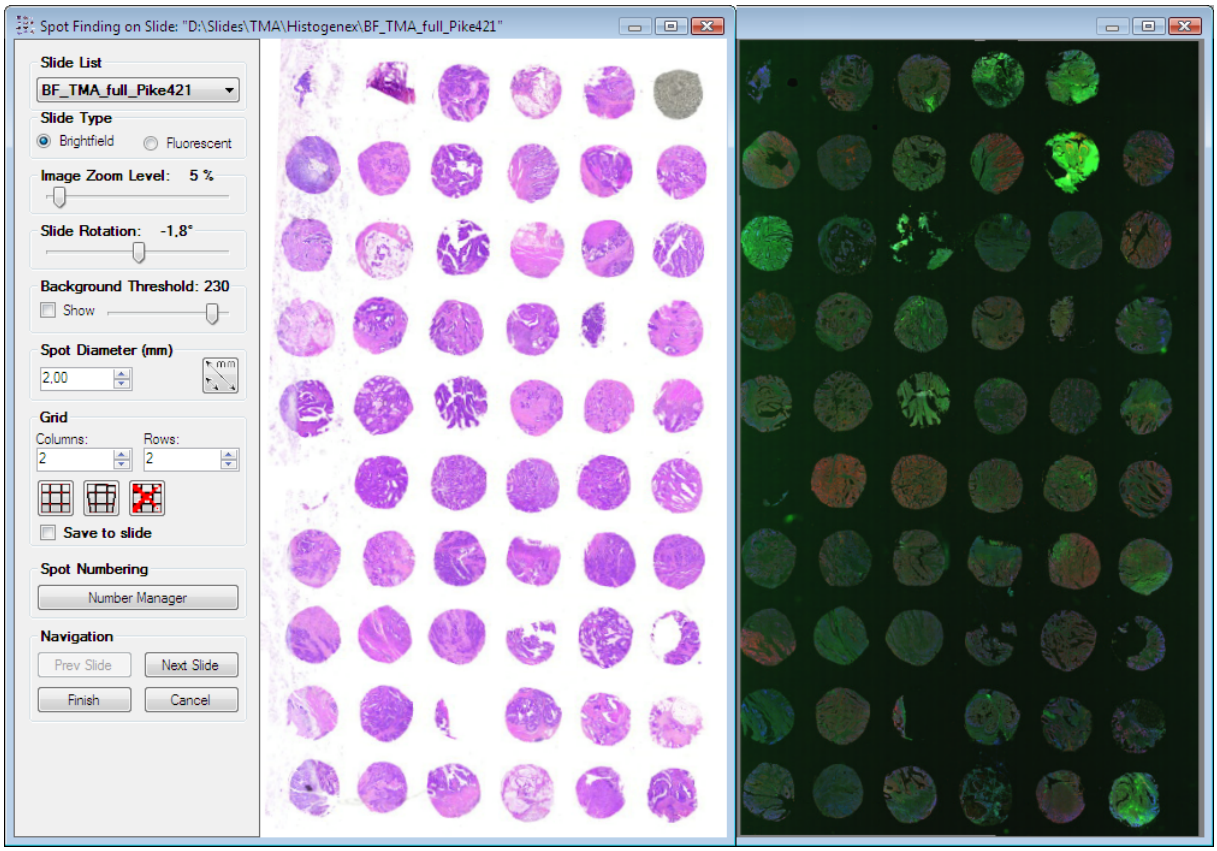
To identify cores and link to patients pathological and clinical data, you must first Import data stored in the Excel TMA database file by choosing the corresponding .XLS file. The software will automatically import all information from the Excel file.

Tip!

TMA Module does not support Excel files newer than 2003 (*.xlsx).

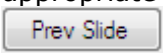
If you have Excel newer than 2003, you must select in **File / Save as..** the following file type: **Excel 97-2003-worksheet (*.xls)**.

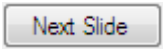
3.9 Finding TMA Spots



Having started a TMA project, the first task is to identify the individual spots on a TMA slide by choosing the Spot Finding option, which opens up a new window. Note that without spot identification no further options of TMA management are accessible. The main function of Spot Finding is to link stained tissue spots to relevant patients data in Excel.

Slide list

The batch mode allows you to access spot finding for all slides in a project in one spot finding window. You do not have to enter spot finding for the slides separately. Select the appropriate slide from the **Slide list** roll-down list. To move between slides, click 

for the previous slide or 

for the next slide in the **Navigation** frame.

Slide Type

Select the appropriate slide type in this frame.

- To apply Spot Finding for a brightfield slide, select **Brightfield**.
- To apply Spot Finding for a fluorescent slide, select **Fluorescent**.

Image Zoom Level

To change the zoom level of the slide, adjust the **Image Zoom Level** slider. The actual zoom level is displayed above the slider.

Slide Rotation

To rotate the slide, adjust the **Slide Rotation** slider. The actual rotation degree is displayed above the slider.

Background threshold

If there is an unwanted background staining, or the specific staining is too weak, you can adjust the **Background threshold** level with the slider to make sure that all stained spots will be scanned. Check the **Show** check box to display the scanned areas according to the threshold level.

Spot Diameter (mm)

To define the diameter of the spots write the correct diameter in millimeters in the text box or set it with the arrows next to the text box. To measure the spot diameter, click
and then pull a line over the largest spot. After releasing the mouse button, the distance will appear in millimeters. Note that the spot diameter parameter has strong effect on the result of spot finding.

Grid

Define the number of spots arranged along the horizontal and vertical axes of the TMA slide (for example 4x6). This can go up to 50x50, but the basis of identification is the same. Enter the correct number of spots in a column in the **Columns** text box and the correct number of spots in the **Rows** text box.

- To draw the grid over the spots, click . Then click the corner spots in the TMA slide one after the other, for example going clockwise. A red X sign will appear on the marked corner spots. After the fourth corner will have been finished, a net of lines will connect the middle of the spots and gray square annotations will surround their borders (the size of square annotations was defined in the Spot Diameter option).

- To remove the grid, click . You can start the procedure again.

- Sometimes spots do not form straight columns and rows due to barrel or other type of distortion. This results in spots being excluded from straight grid lines.

  This problem can be solved by grid retorsion. Click to move grid elements on spots.

  - *To align grid elements to whole rows or columns* hold the left mouse button on the the row / column line and drag it with the mouse. When activated, the centers of the given grid row / column becomes red.

  - *To align a single grid element*, drag not the line (as above), but the dot itself. This time only one single dot will become red.

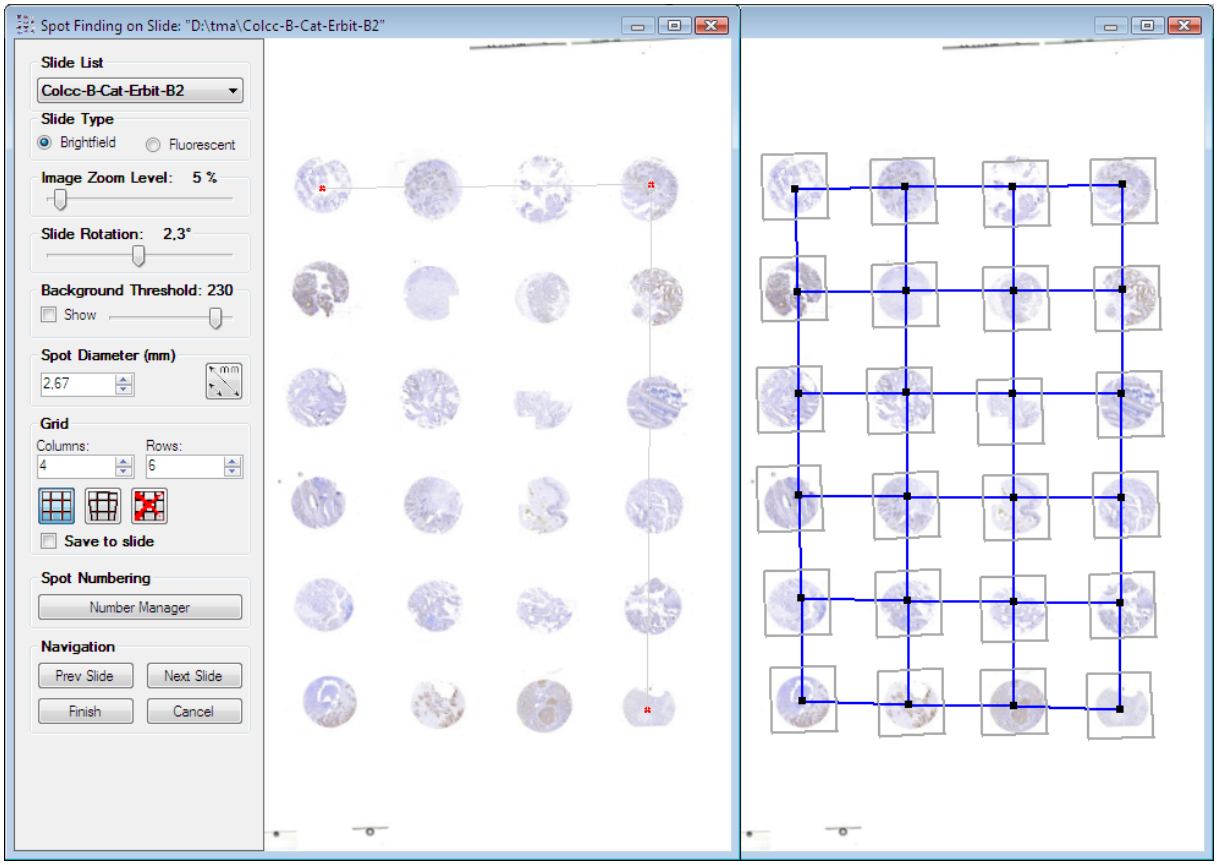
  - If the above steps do not help fitting the grid on the spots (for example because it detects specks as specimens), you can also *align the grids to the spots manually*. To do this, hold the right mouse button on the center dot of one grid element and drag it to the required position.

  - If you *delete a grid* (in the case of multiple grids existing on a slide), the grid of the last spot group will be active.

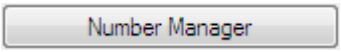
In spot finding, the software stores the last threshold value, spot diameter and the grid dimension used.

If you generate several grids for several spot groups in a slide, you can activate those by clicking on the grid, inside the grid, or on the spot frame. When activated, the grid color turns blue from gray.

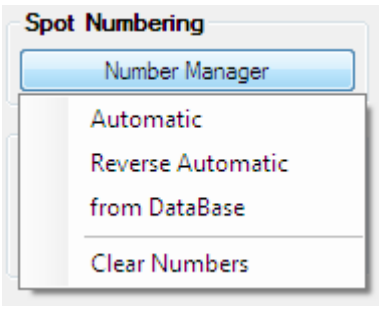
Select the **Save to slide** check box if you want to make measurements for individual spots in the different Quant applications. These options are only active if you save grids into the slide. For the TMA scoring, it is indifferent whether you choose to save grids to slide or not.



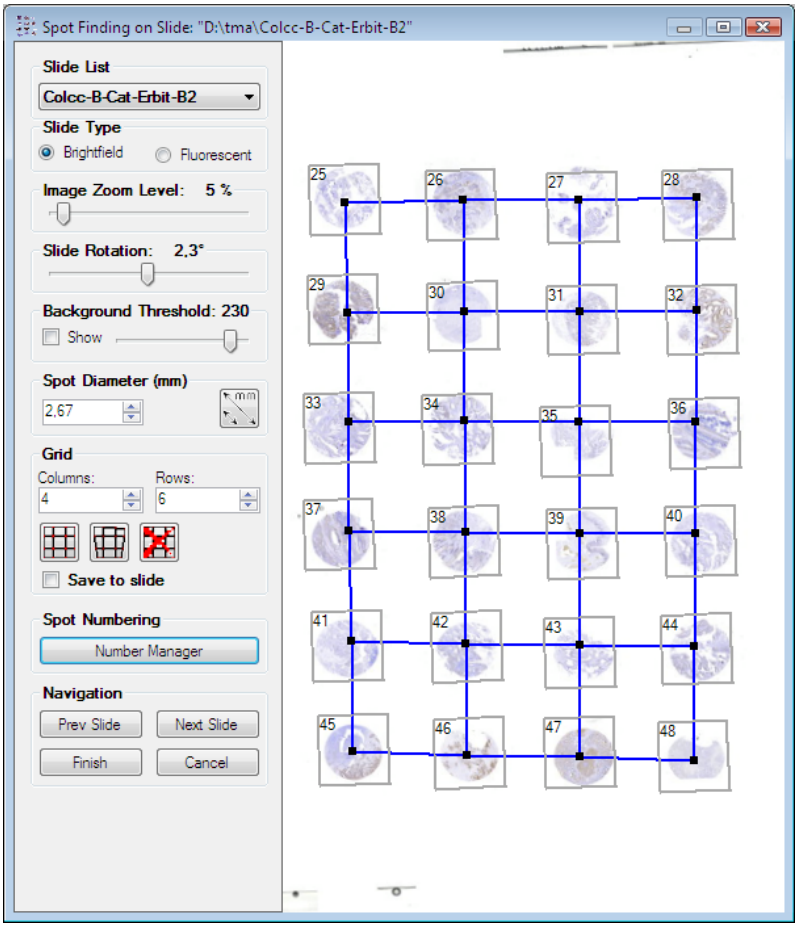
Spot numbering

To access the Numbering menu, click 

.

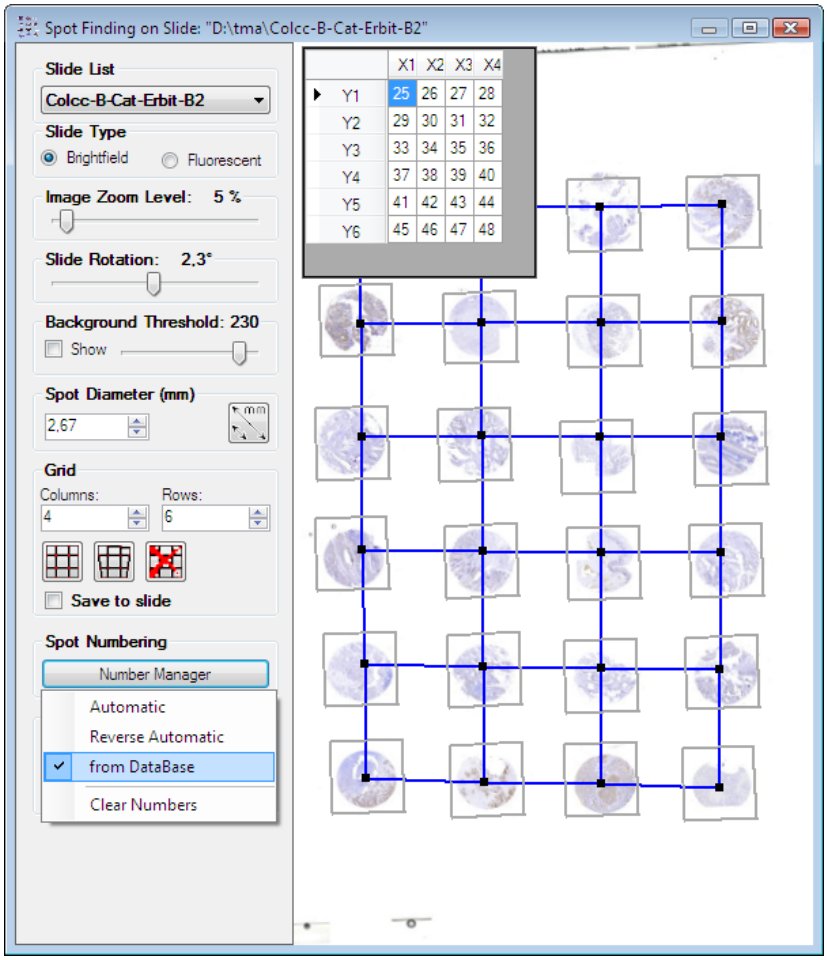


- Select the **Automatic** option, if the spot map is regular without any rows or column of spots missing and the numbering starts from the upper left corner of a TMA slide.



- For reverse order numbering, select the **Reverse Automatic** option.

- Select the **from DataBase** option to open a window showing the original spot map from the Excel database file (Block1, Block2, and so on…). Identification of the spots can be done by clicking on one of the corner numbers in the Excel spot map and dragging it over the corresponding stained spot (drag-and-drop). Having repeated this by pulling over the number from the uppermost opposite corner and placing it to the opposite corner of the TMA slide will result in the desired numbering to appear over the cores.



Important!

Using an orientation tissue easily differentiated from the test tissue, such as liver (as suggested above) for example when testing breast cancer samples, safely assists in the accurate core finding. However, this is optional and any other system may be used for recognizing TMA slide orientation.

- To cancel the grid-numbering such as the spot numbers, select the **Clear Numbers** option.

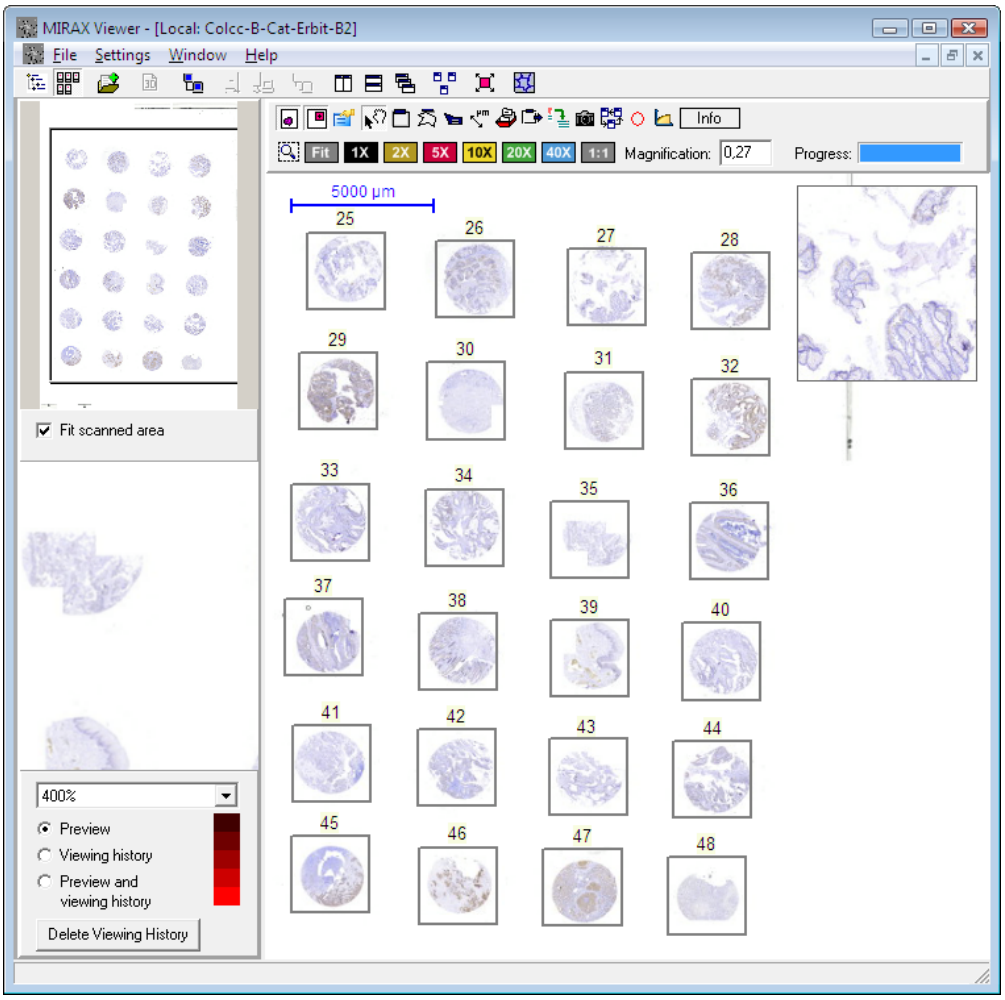
Navigation

- To move between slides, click Prev Slide for the previous slide or Next Slide for the next slide.

- Click Cancel to cancel the spot finding process.
- After spot identification layout will have been accepted, the annotations and numbers attached will be saved by clicking Finish.

3.10 Adjusting Annotations in the MIRAX Viewer 1.12

Identified TMA slides can be opened in the MIRAX Viewer 1.12 software either by double clicking on their preview slide or by using the right mouse button and choosing Open Slide option from the pop up menu. The TMA identifier square linked with the core ID number annotation will appear on each stained tissue core if you have selected the **Save to Slide** option in the **Spot Finding** window. If there is no such annotation seen, the TMA slide needs to be identified by using the Spot Finding function.



4 Scoring

The latest version of TMA software allows for using different scoring schemes within the same project for example when markers detected (stains) in a project cannot be assessed on the same basis. The schemes are stored and offered for later use under the Stain option.

The name and value of empirical scores used in a project can be predefined in the Scoring Scheme menu.

The first step is to chose the stain (in the Stains panel, from combo box Selected Stain) for defining the score-values.

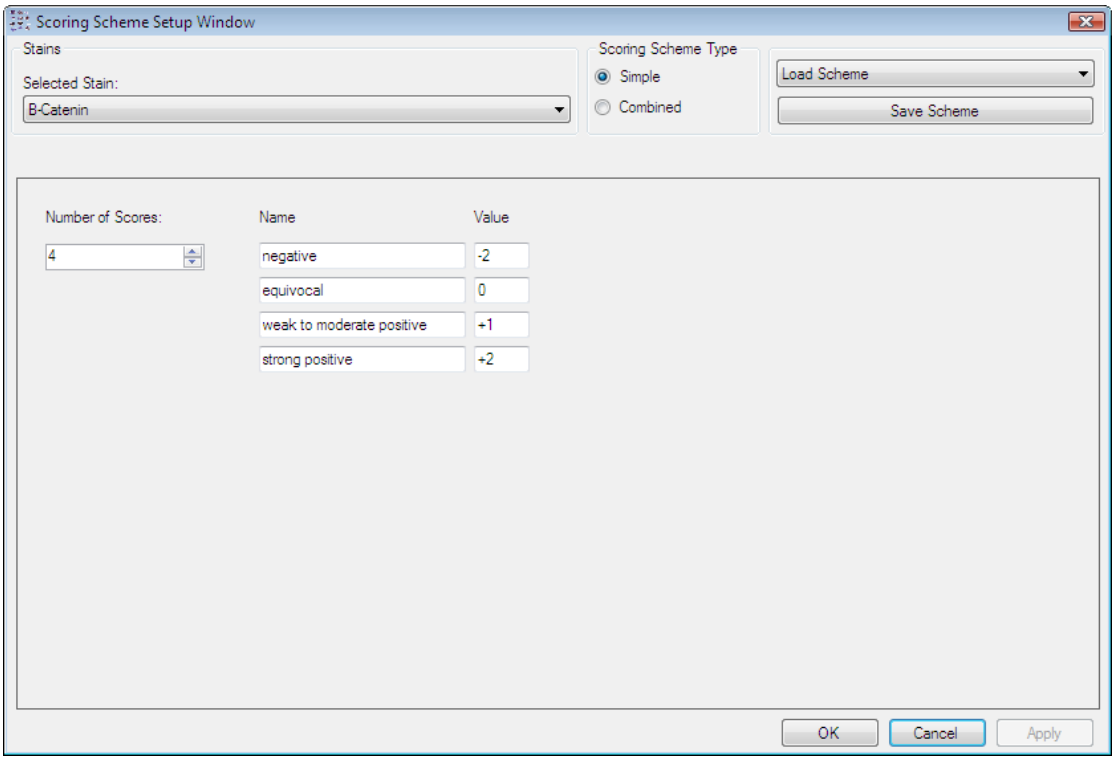
The second step is to chose from options Simple or Combined in the Scoring Scheme Type panel.

4.1 Scoring Setup

4.1.1 Simple Scoring Scheme

Select the Simple option in the Scoring Scheme Type panel.

Scoring can be defined on a maximum 10-scale system allowing for considering staining intensity or % of positive cells. To change the number of scores, click the Up or Down arrow buttons.

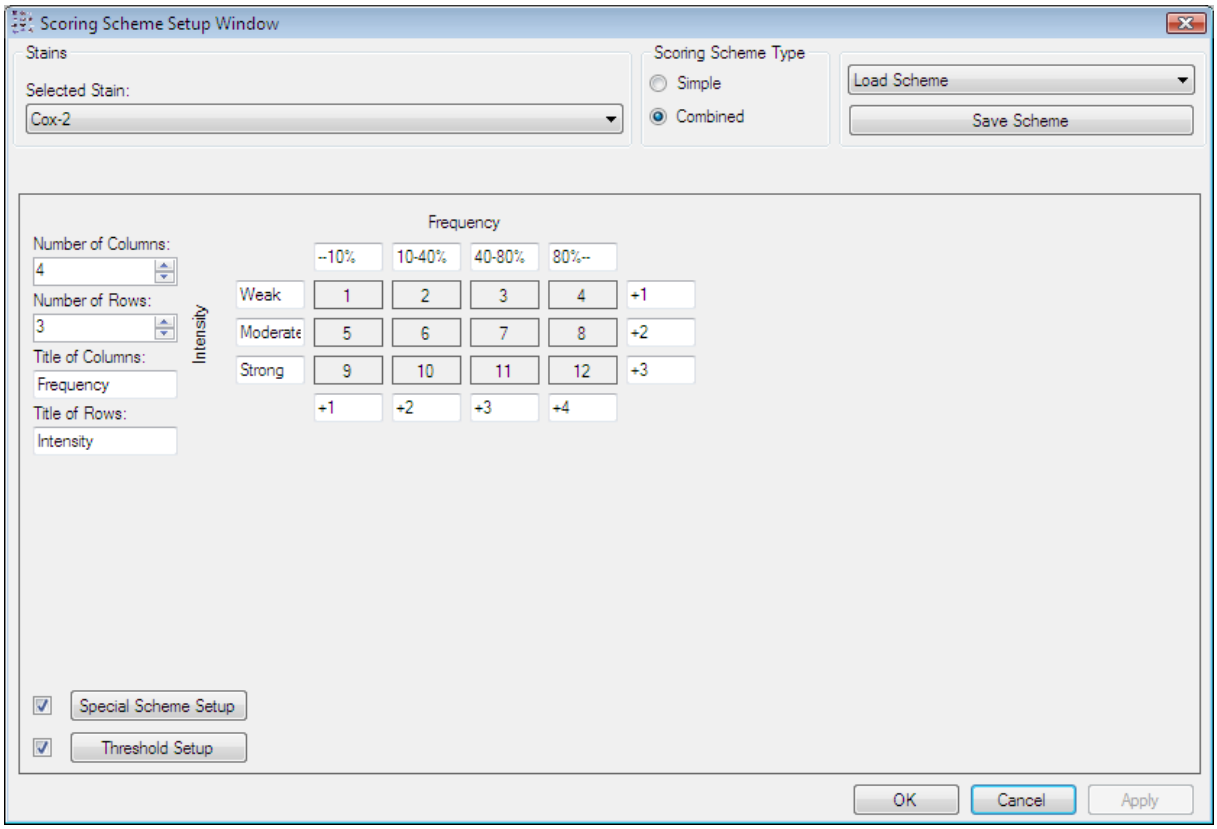


Though options are flexible and there are many, the use of a 4-scale system including -2 = negative, 0 = equivocal, 1 = weak-moderate positive, 2 = strongly positive scores, is practical for later Unsupervised Hierarchical Clustering, which may be done without using score conversion (Deconvoluter).

4.1.2 Combined Scoring Scheme

Select the Combined option in the Scoring Scheme Type panel.

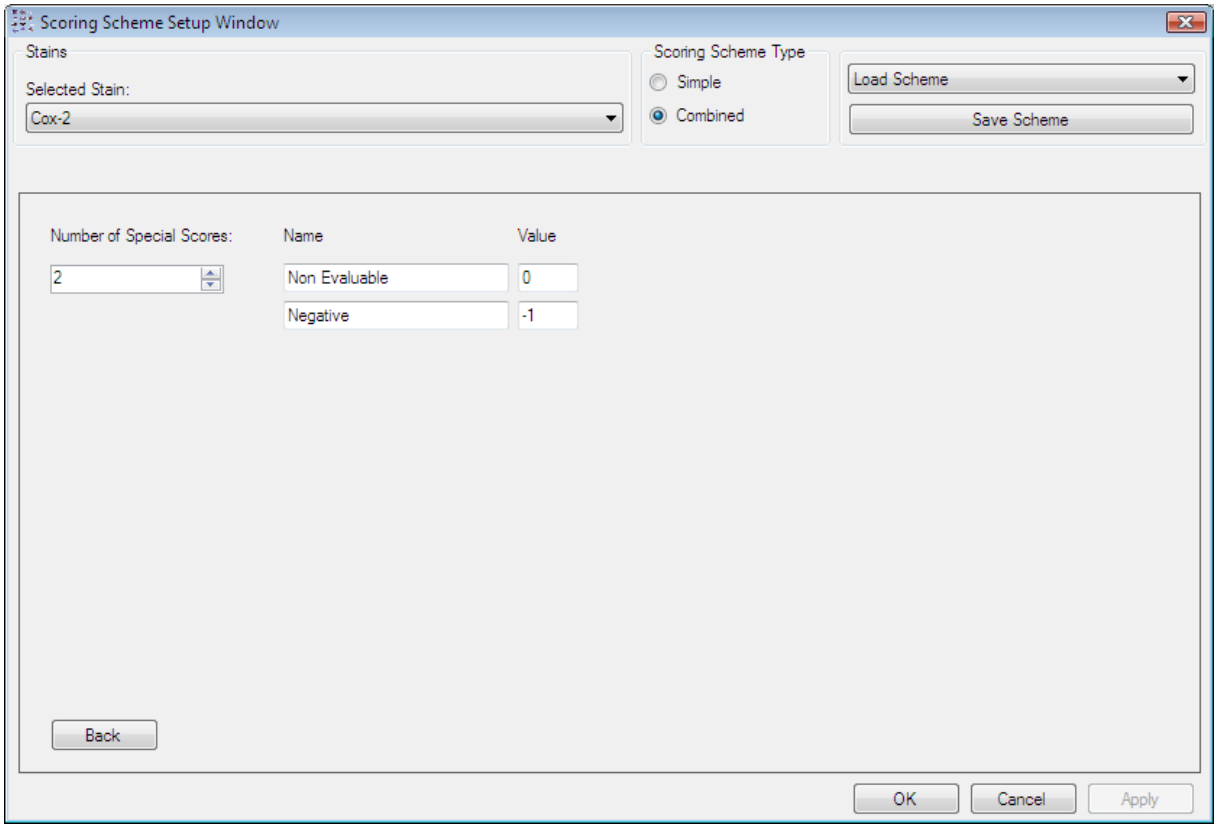
Either enter or select the property you want to evaluate and then define the number of rows and columns (max. 10 rows and 10 columns). Then enter either the ranges of the chosen two properties or the structural-functional parts of the cell (for Frequency-values, for example, 0-10 %, 1-40 %, 40-80 %, 80-100 %; for Stain-Intensity: weak, moderate, strong; for Localisation: nucleus, cytoplasm, membrane) into the heads of rows and columns.



Then enter the score-value into each end-field (text-box) of rows and columns.

Making Special Schemes:

You can define \ name special schemes giving them a value, a number (for example, 0 = Non Evaluable; -1 = Negative) after marking in the Special Scheme Setup option.

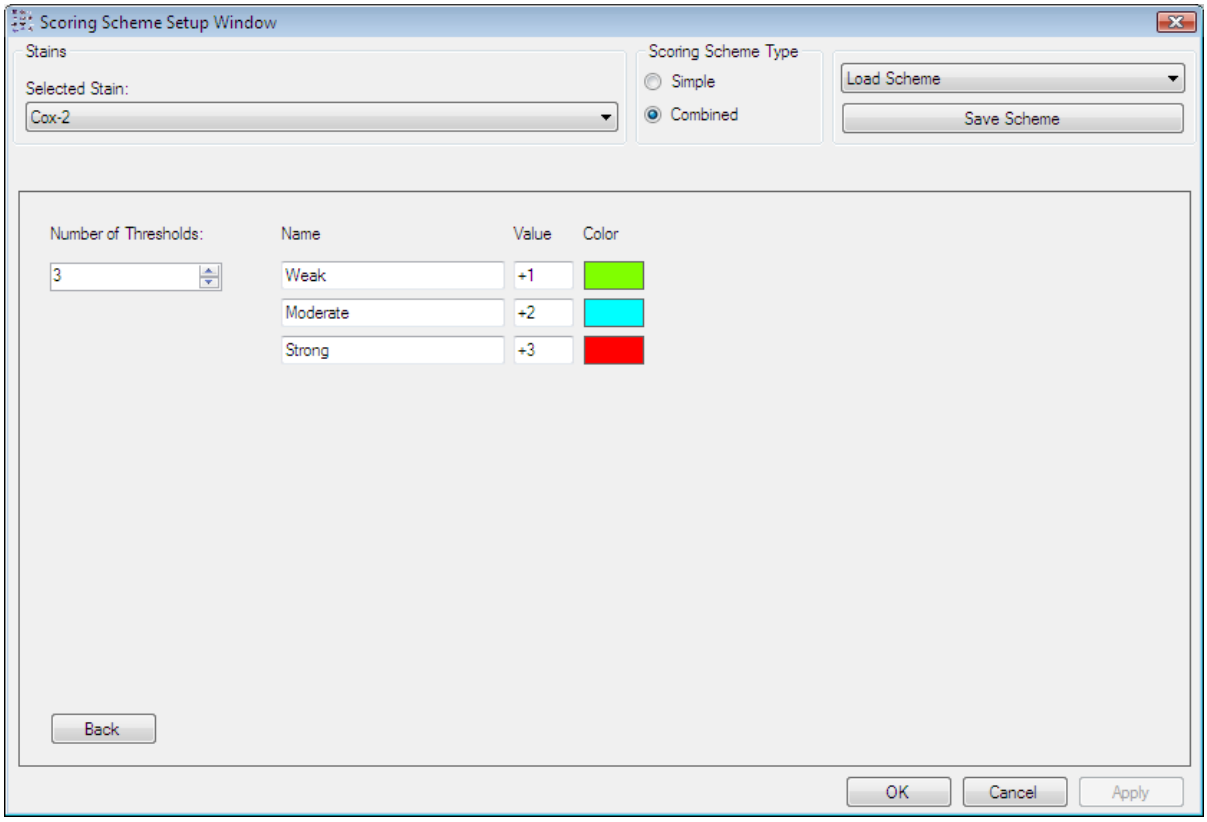


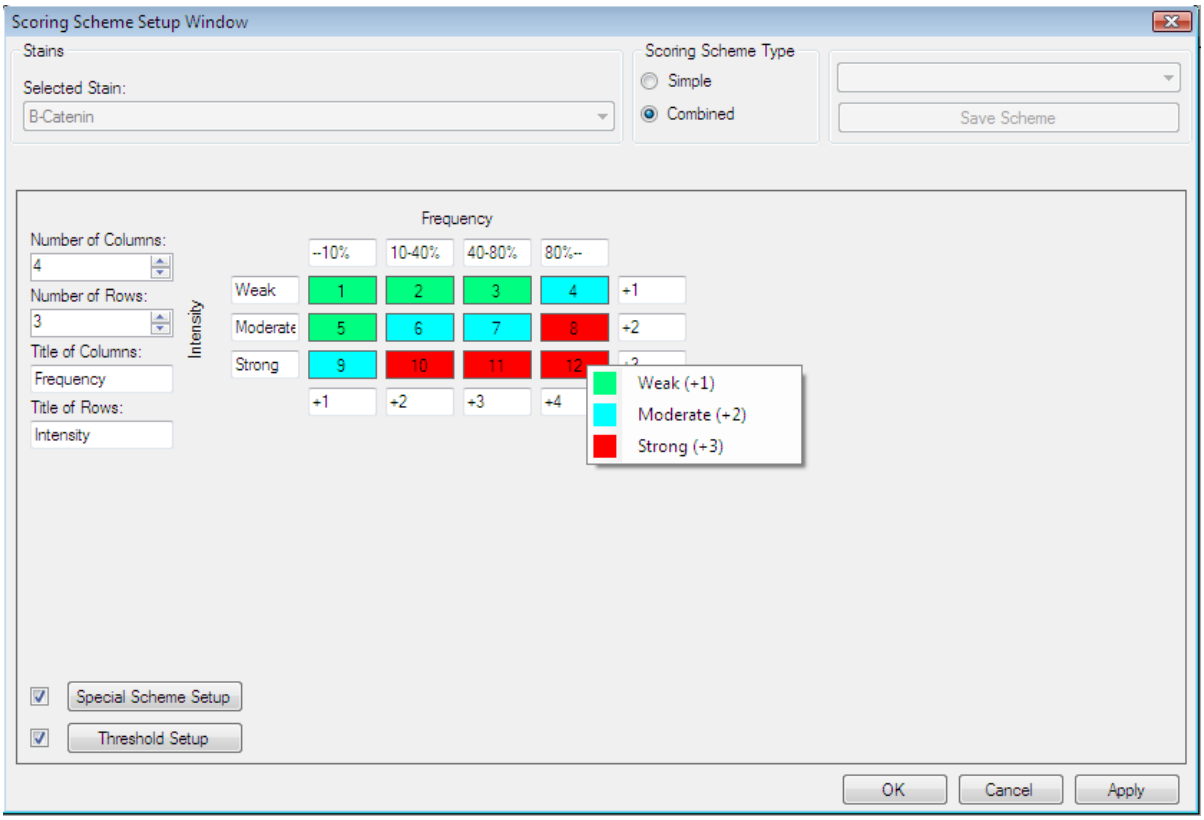
To return to the Scoring Scheme menu, click **Back**.

Threshold Setup:

After marking in the Threshold Setup option, you can define groups of scoring schemes for showing a common, visually hidden but known by the researcher, biological property of these value-groups (for example, a given DNA-section produces the examined protein in various rows and columns).

You can define the number of Clusters, and you can name these Clusters, writing a value into the text boxes of these threshold-groups, and giving a chosen color (by right mouse clicking on color box under the Color option).


Scoring Scheme Setup Window
Stains
Selected Stain:
Cox-2
Scoring Scheme Type
Simple
Combined
Load Scheme
Save Scheme
Number of Thresholds:
3
Name
Value
Color
Weak
+1
Moderate
+2
Strong
+3
Back
OK
Cancel
Apply


Scoring Scheme Setup Window
Stains
Selected Stain:
B-Catenin
Scoring Scheme Type
Simple
Combined
Save Scheme
Frequency
Number of Columns:
4
Number of Rows:
3
Title of Columns:
Frequency
Title of Rows:
Intensity
--10%
10-40%
40-80%
80%--
Weak
Moderate
Strong
1
2
3
4
5
6
7
8
9
10
11
12
+1
+2
+3
+4
Weak (+1)
Moderate (+2)
Strong (+3)
Special Scheme Setup
Threshold Setup
OK
Cancel
Apply

To return to the Scoring Scheme menu, click **Back**.

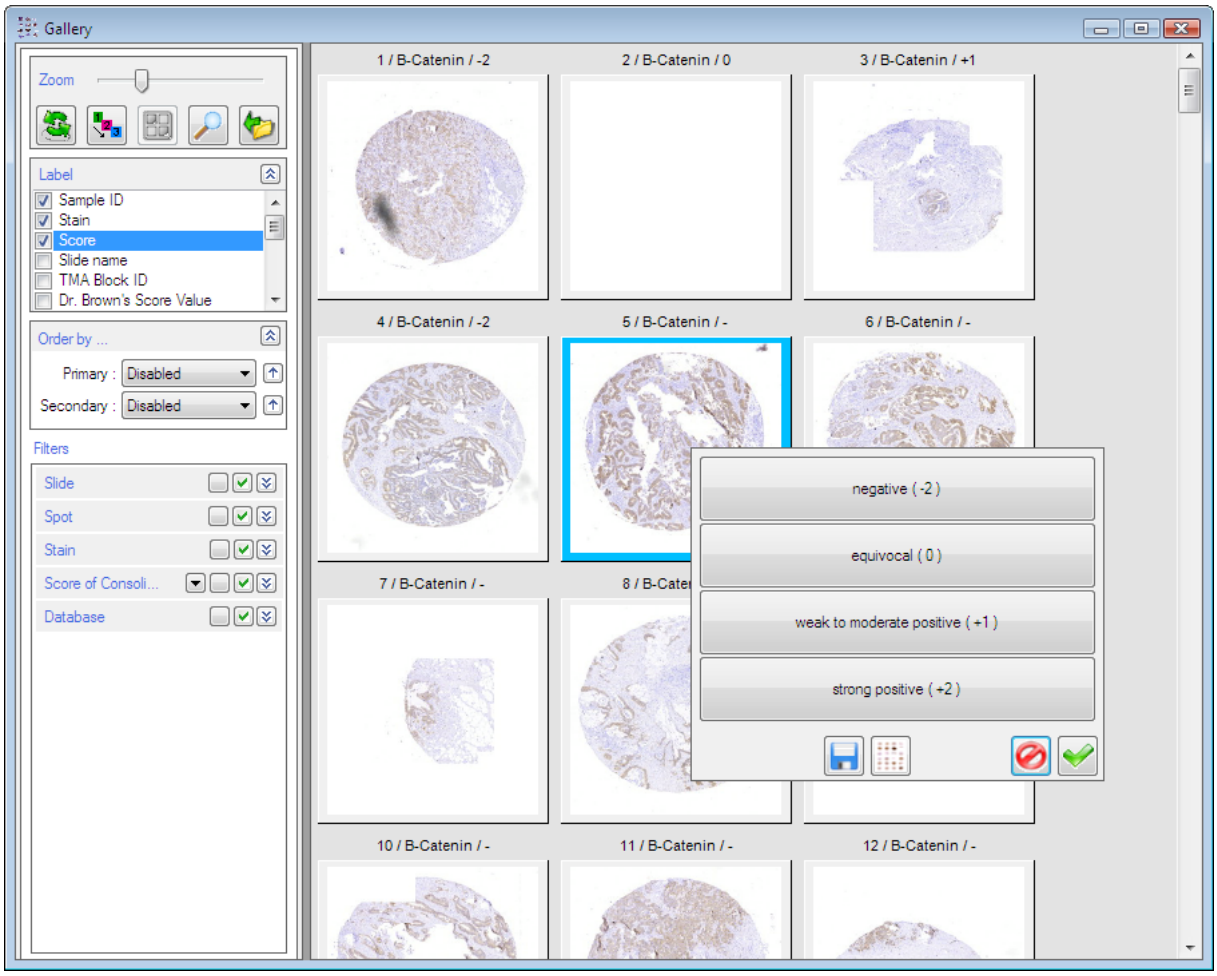
After all these steps, click **Apply** or **OK**.

4.2 Scoring TMA Spots

Scoring of stained TMA spots can be done from the Gallery. Scoring from Gallery offers much more flexibility in this updated version (see later), thus it should be preferred for scoring. Validated scoring - allowing for possible re-scoring of spots in Gallery mode when sorted with other spots along with any common feature(s) - is an important function of the MIRAX TMA Module software, ensuring reproducible results without the risk of false conclusions based on inconsistent scoring.

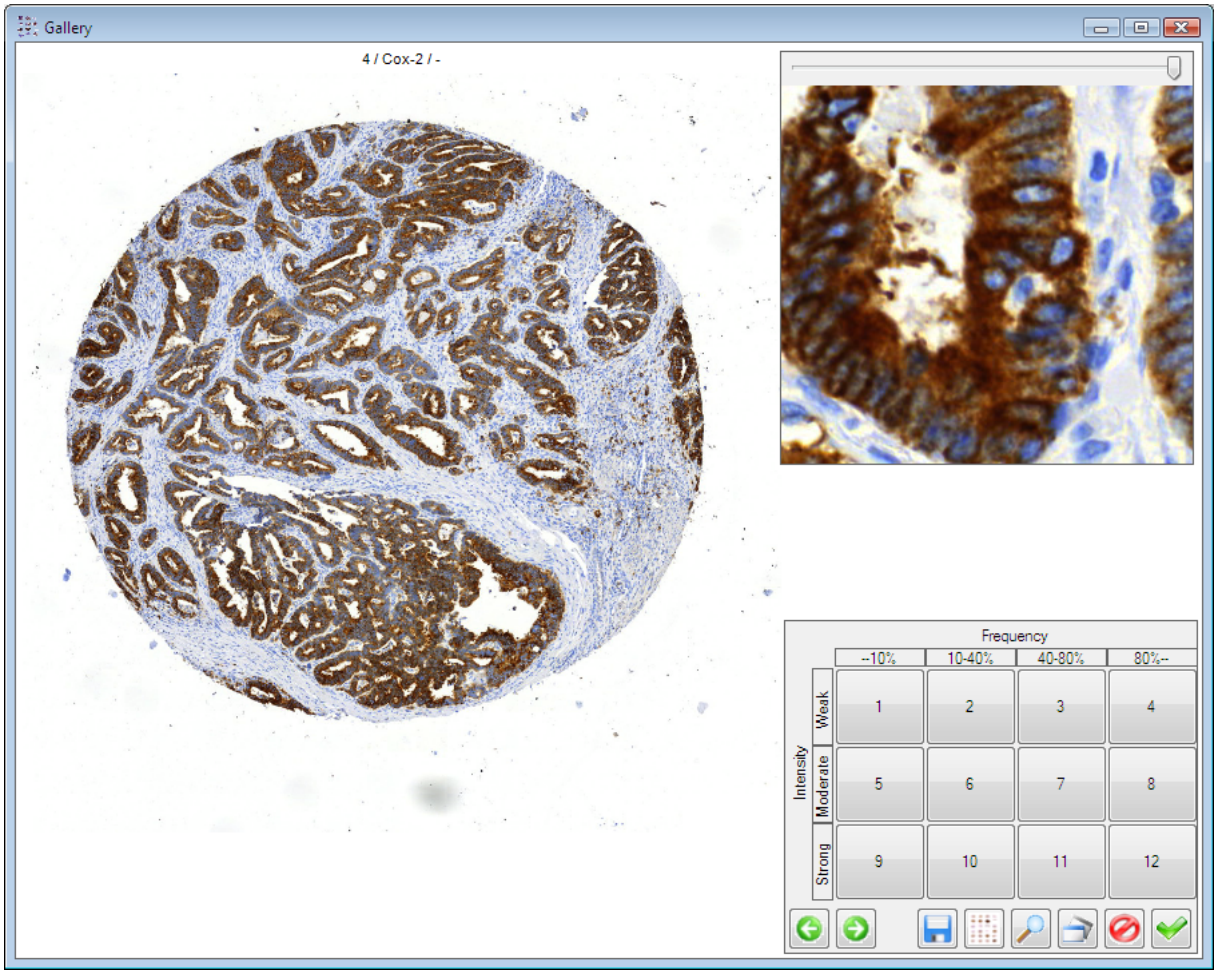
4.2.1 Scoring in Gallery

The scoring can be done by clicking on the spot with the right mouse button and choosing a score from the pre-defined values.



If a score has already been attached it is indicated by a pushed-down button in the scoring panel and the score appears in the label above the spot after spot ID and stain.

4.2.2 Sequential Scoring



Sequential scoring in the Gallery automatically offers full size image of a spot on the screen, which can be further magnified in the magnifying window. Scored image will disappear allowing for the next spot to be displayed for scoring.

4.3 Consensus Autoscore

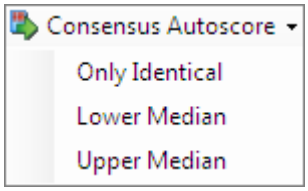
In case of more than one assessors working in a project, the Consolidate option is automatically offered, which can be used as a separate scorer (user) window for creating consensus scores.

Consensus Autoscore function allows automated consolidation of scores by accepting values identical at all assessors of by choosing the median values of all scorers to be

registered into the score boxes of the consolidator.

All scoring data are accessible and can be used for filtering in the Gallery.

The Score label represents the person working under the Consolidate option. The order of scores given by different users seen above the gallery spots is the same as their order under the Labels option (left hand panel).

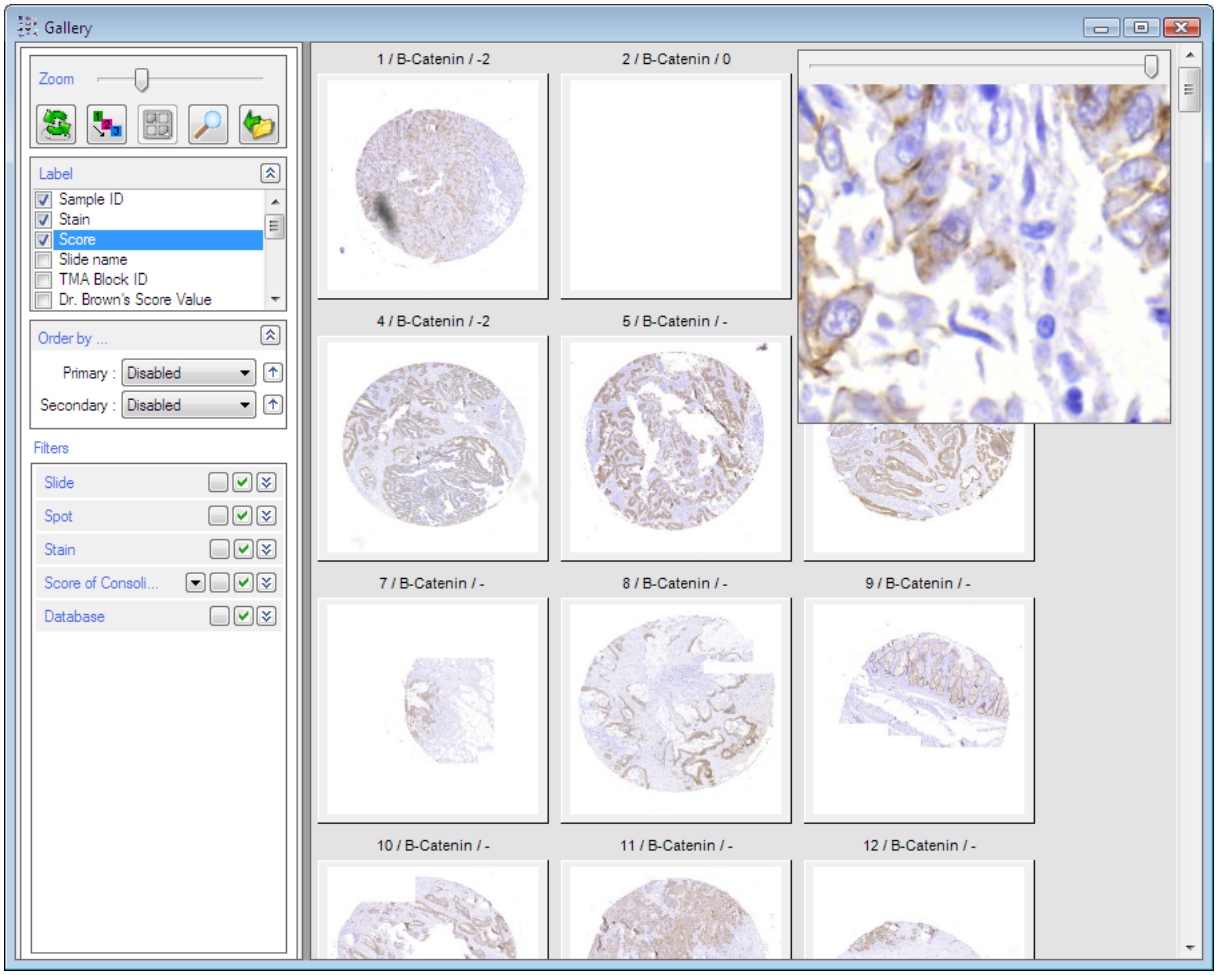


5 Managing Spots in the Gallery

In the Gallery, stained TMA spots can be sorted into restricted groups irrespective of their physical location (for example different glass slides). The order of the spots can be defined according to their staining, score values, slide names, spot IDs and parameters which were defined in the database listed under the Filters option (lower left panel). You can arrange the gallery based on primer and seconder features.

Important!

After changing settings, click to update the gallery.



5.1 Function Buttons

(In the left upper window-corner)

Desired spot magnifications can be set up with the Zoom panel to fit on the screen.

There are 5 function buttons below the Zoom option.

- To initiate gallery spot collection, click . Click this button every time after you have changed the conditions to update the gallery.
- To allow sequential scoring, click .
- To allow saving of the selected image-collection, click .

- To display the spots in their original position on the slides, click (matrix view). This function is only active if you have filtered for only one slide, or you are using a one-slide project.

- To activate the gallery zoom window, click .

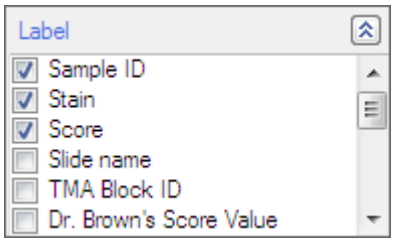
  - To position the gallery zoom window, click on the window with the left mouse button and drag it to the desired position.

  - To shrink or enlarge it, use the mouse scroll wheel.

  - To zoom in or out, use the scroll bar.

5.2 Labels

(Below the function buttons)



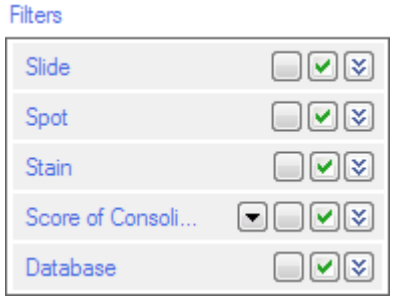
From the Labels panel one can select what information to be shown above the spots in the gallery. There can be as many as the number of patients related feature groups (columns) in the Excel Lookup table.

Options in the Filters panel (lower left) allow for creating selected spot collections based on the listed features alone or combined. The default of each parameter is All, for selecting all members of the given category. Each filter parameter group is defined by one column in the Excel Lookup table and most of them are optional.

To check whether a spot has been scored or not, check the Score check box. If the spot has been scored, the score number is displayed. If not, a – character is displayed.

5.3 Filter Parameters

5.3.1 Filtering For All Users



Sample ID correspond to the Unique TMA Sample ID in Excel. In some cases one sample ID identifies one patient. However, in most cases, particularly at tumor heterogeneity, or small core diameter, more than one sample (2-5) of a patient may be used, which can be managed under different sample IDs and grouped into gallery by sorting together in the Gallery with donor block ID. Restricting Sample ID to one sample only and leaving all other parameters at their default (All) allows for the rapid confirmation of the accuracy of core identification.

Restricting the Score and Stain options into one parameter each will allow comparing spots of a particular staining obtained the same score. The inconsistently scored samples can be recored within the Gallery function before final exporting of the results into Excel database for further correlation studies (validated scoring). This feature of the MIRAX TMA Module allows for using highly reliable score values for statistical-, survival- and cluster analyses.

In the Gallery, the spots which have not yet been scored can be collected with filtering. Scored (on the base of the Special Scheme in the Scoring Scheme Setup menu) spots can be re-selected and re-scored. In equivocal cases, doubtfully scored spots can be reseted for non-scored, allowing their re-scoring at the end of a project based on a professional consultation, when collected them together.

The space for gallery images can be extended full screen on the expense of the left hand gallery menu panel by clicking on dark gray bar in the right edge of gallery menu panel.

To filter based on the Excel database, select the Database filter.

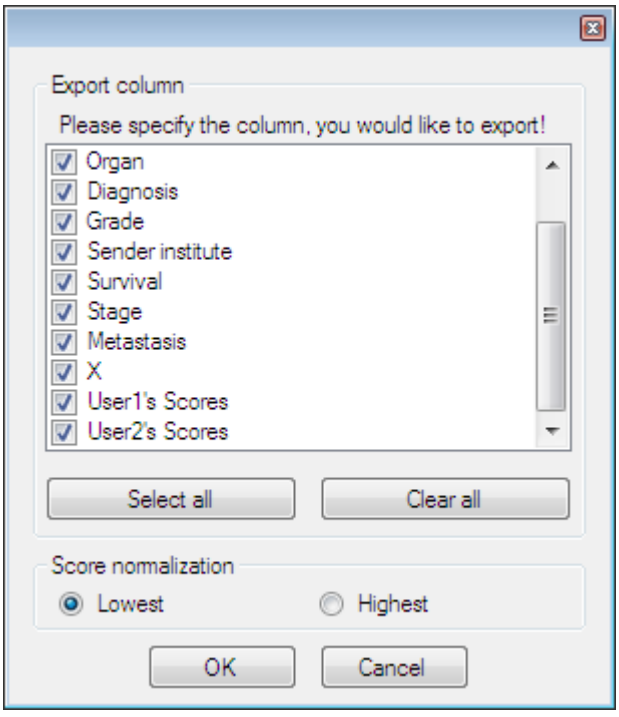
5.3.2 Filtering Only For Consolidate User

Only the Consolidate User has a chance to assembling spot-galleries on the base of arbitrarily selected scores of all users.

1. Click the button next to the Score of Consolidate text.
2. A menu is displayed where you can select the other users.
3. After selecting the users, you can select the scores.
4. After this, you can select the appropriate filtering options. For example: Not Scored, "+2" at user1 and "+1" at user2. In this case, the Gallery will only include those spots that satisfy these conditions.

6 Exporting Data and Images

6.1 Exporting Data



When exporting, select which Database column(s) you would like to export.

If you are signed in as a Consolidate user, you can also export the scores of other users. In this case, select the names of the users whose scores you would like to export.

Score normalization

There can be slides, where more spots are included from the same donor. These spots are usually identified with the same Core ID. However, the Excel sheet has only 1 row for 1 ID, therefore it has to be decided which score should the software include in that row.

In this case, the software will calculate the median of all the scores depending on whether the Lowest or the Highest option was selected.

Warning!

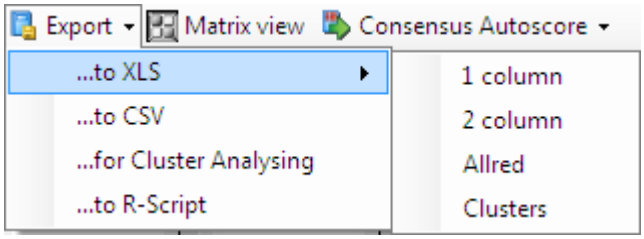
TMA Module does not support the newer than 2003 Excel files (*.xlsx).

If you have Excel newer than 2003, you must know that although **the file-extension will be .xls but the file structure will be according to .xlsx file extension**.

If you have Excel newer than 2003, you must select in **File / Save as..** the following file type: **Excel 97-2003-worksheet (*.xls)**.

After finishing scoring the database including all imported data of the patients added with the scores of the TMA spots can be exported into either a new Excel file (XLS) or a CSV format, by choosing the Export option from the project menu.

Having chosen the Combined option in the Scoring Scheme Type panel, the user has got the following possibilities for exportation (click Export, then select XLS option).



6.1.1 Exporting Score Values to 1 Column

It makes possible to save only one Score Value to each spot.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Unique TMA Sample ID	TMA Block ID	Donor Block ID	Organ	Diagnosis	Grade	nder instit	Survival	Stage	B-Catenin	Cox-2	EGFR-Ventana	p53-SP5
2	1	Block1	1722-02	liver	cirrhosis	G0	Szombathely			-2	7	+2	3
3	2	Block1	546-04	breast	IDC	G2	Bay			+1	11	+9	6
4	3	Block1	546-04	breast	IDC	G2	Bay			+1	7	+9	9
5	4	Block1	210-04	breast	Mucinosus	G2	Bay			+2	3	+7	10
6	5	Block1	210-05	breast	IDC	G3	Bay			+1	7	+2	13
7	6	Block1	1643-04	breast	IDC	G3	Bay			+2	3	+1	7
8	7	Block1	1416-04	breast	ILC	G2	Bay			+1	3	+2	12
9	8	Block1	1610-04	breast	IDC, come	G2	Bay			0	6	+1	16
10	9	Block1	128-05	breast	ADC meta	poor	Bay			+1	9	0	10
11	10	Block1	774-02	breast	connective	G0	S.E. I. Pathology			0	5	-2	10
12	11	Block1	659-02	breast	ILC	G3	S.E. I. Pathology			-2	10	+7	5
13	12	Block1	322-02	breast	IDC	G2	S.E. I. Pathology			0		+2	1
14	13	Block1	1315-02	breast	IDC	G2	S.E. I. Pathology			-2	0	0	3
15	14	Block1	1199-02	breast	IDC	G3	S.E. I. Pathology			0		+1	6
16	15	Block1	4761-02	breast	DCIS	G0	S.E. I. Pathology			+1	-1	+9	8
17	16	Block1	657-03	breast	connective	G0	Bay			+2	8	+7	
18	17	Block1	1222-03	breast	IDC	G2	Bay			+1	4	-2	10
19	18	Block1	1324-03	breast	ILC	G1	Bay			+2	2	0	16
20	19	Block1	1643-04	breast	IDC	G2	Bay			+1	1	+1	11
21	20	Block1	1643-04	breast	IDC	G2	Bay			0	10	+9	2
22	21	Block1	1826-02	breast	DCIS	G0	S.E. I. Pathology			+1	6	+7	9
23	22	Block1	1877-02	breast	ILC	G2	Natl. Inst. Cancer			0	7	+2	14
24	23	Block1	988-03	breast	IDC	G2	Bay			+1	3	+1	4
25	24	Block1	1023-03	breast	IDC	G0	Bay			0	2	+2	8
26	25	Block2	210-05	breast	connective	G3	Bay			+1	6	0	11
27	26	Block2	1643-04	breast	ILC	G2	Bay			0	11	-2	10
28	27	Block2	1416-04	breast	IDC	G2	S.E. I. Pathology			0	12	+1	6
29	28	Block2	1610-04	breast	IDC	G3	S.E. I. Pathology			0	8	+7	1
30	29	Block2	128-05	breast	IDC	G3	S.E. I. Pathology			0	12	+1	11
31	30	Block2	774-02	breast	DCIS	G2	S.E. I. Pathology			0	5	0	15
32	31	Block2	1315-02	breast	connective	G2	S.E. I. Pathology			-2	9	-2	16

6.1.2 Exporting Score Values to 2 Columns

It makes possible to save the Score Values of a given spot recorded on a 2D-scale into two separated columns.

This export type is only valid if the slide was scored with a Combined Scoring Scheme.

	A	B	C	D	E	F	G	H	I	J	K	
1	Unique TMA Sample ID	TMA Block ID	Donor Block ID	Organ	Diagnosis	Grade	nder instit	Survival	B-Catenin	Cox-2_Frequency	Cox-2_Intensity	EGFR
2	1	Block1	1722-02	liver	cirrhosis	G0	Szombathely		-2	+3	+2	+2
3	2	Block1	546-04	breast	IDC	G2	Bay		+1	+3	+3	+9
4	3	Block1	546-04	breast	IDC	G2	Bay		+1	+3	+2	+9
5	4	Block1	210-04	breast	Mucinosus	G2	Bay		+2	+3	+1	+7
6	5	Block1	210-05	breast	IDC	G3	Bay		+1	+3	+2	+2
7	6	Block1	1643-04	breast	IDC	G3	Bay		+2	+3	+1	+1
8	7	Block1	1416-04	breast	ILC	G2	Bay		+1	+3	+1	+2
9	8	Block1	1610-04	breast	IDC, come	G2	Bay		0	+2	+2	+1
10	9	Block1	128-05	breast	ADC meta	poor	Bay		+1	+1	+3	0
11	10	Block1	774-02	breast	connective	G0	S.E. I. Pathology		0	+1	+2	-2
12	11	Block1	659-02	breast	ILC	G3	S.E. I. Pathology		-2	+2	+3	+7
13	12	Block1	322-02	breast	IDC	G2	S.E. I. Pathology		0			+2
14	13	Block1	1315-02	breast	IDC	G2	S.E. I. Pathology		-2	0	0	0
15	14	Block1	1199-02	breast	IDC	G3	S.E. I. Pathology		0			+1
16	15	Block1	4761-02	breast	DCIS	G0	S.E. I. Pathology		+1	-1	-1	+9
17	16	Block1	657-03	breast	connective	G0	Bay		+2	+4	+2	
18	17	Block1	1222-03	breast	IDC	G2	Bay		+1	+4	+1	-2
19	18	Block1	1324-03	breast	ILC	G1	Bay		+2	+2	+1	0
20	19	Block1	1643-04	breast	IDC	G2	Bay		+1	+1	+1	+1
21	20	Block1	1643-04	breast	IDC	G2	Bay		0	+2	+3	+9
22	21	Block1	1826-02	breast	DCIS	G0	S.E. I. Pathology		+1	+2	+2	+7
23	22	Block1	1877-02	breast	ILC	G2	Natl. Inst. Cancer		0	+3	+2	+2
24	23	Block1	988-03	breast	IDC	G2	Bay		+1	+3	+1	+1
25	24	Block1	1023-03	breast	IDC	G0	Bay		0	+2	+1	+2
26	25	Block2	210-05	breast	connective	G3	Bay		+1	+2	+2	0
27	26	Block2	1643-04	breast	ILC	G2	Bay		0	+3	+3	-2
28	27	Block2	1416-04	breast	IDC	G2	S.E. I. Pathology		0	+4	+3	+1
29	28	Block2	1610-04	breast	IDC	G3	S.E. I. Pathology		0	+4	+2	+7
30	29	Block2	128-05	breast	IDC	G3	S.E. I. Pathology		0	+4	+3	+1
31	30	Block2	774-02	breast	DCIS	G2	S.E. I. Pathology		0	+1	+2	0
32	31	Block2	1315-02	breast	connective	G2	S.E. I. Pathology		-2	+1	+3	-2
33	32	Block2	1199-02	breast	IDC	poor	S.E. I. Pathology		0	+2	+1	+2

6.1.3 Exporting Score Values for Generating Allred Values

The software generates an Allred Value from the Score Value given by the user.

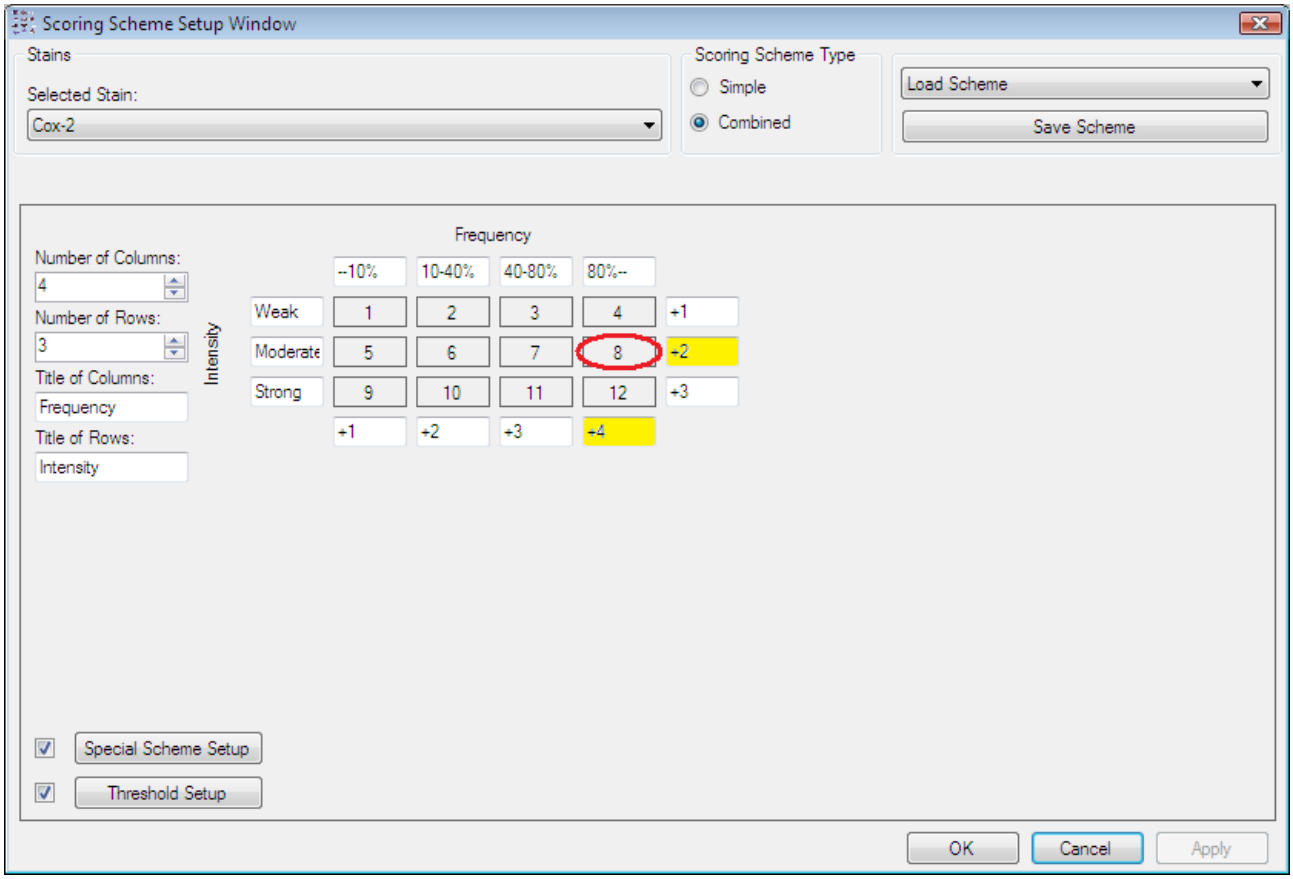
The Allred Scoring Scheme is preferred mainly in the breast cancer diagnosis.

This export type is only valid if the slide was scored with a Combined Scoring Scheme.

	A	B	C	D	E	F	G	H	I	J	K
1	Unique TMA Sample ID	TMA Block ID	Donor Block ID	Organ	Diagnosis	Grade	nder instit	Survival	B-Catenin	Cox-2_AllRed	EGFR-Ventana
2	1	Block1	1722-02	liver	cirrhosis	G0	Szombathely		-2	5	+2
3	2	Block1	546-04	breast	IDC	G2	Bay		+1	6	+9
4	3	Block1	546-04	breast	IDC	G2	Bay		+1	5	+9
5	4	Block1	210-04	breast	Mucinosus	G2	Bay		+2	4	+7
6	5	Block1	210-05	breast	IDC	G3	Bay		+1	5	+2
7	6	Block1	1643-04	breast	IDC	G3	Bay		+2	4	+1
8	7	Block1	1416-04	breast	ILC	G2	Bay		+1	4	+2
9	8	Block1	1610-04	breast	IDC, come	G2	Bay		0	4	+1
10	9	Block1	128-05	breast	ADC meta	poor	Bay		+1	4	0
11	10	Block1	774-02	breast	connective	G0	S.E. I. Pathology		0	3	-2
12	11	Block1	659-02	breast	ILC	G3	S.E. I. Pathology		-2	5	+7
13	12	Block1	322-02	breast	IDC	G2	S.E. I. Pathology		0		+2
14	13	Block1	1315-02	breast	IDC	G2	S.E. I. Pathology		-2	0	0
15	14	Block1	1199-02	breast	IDC	G3	S.E. I. Pathology		0		+1
16	15	Block1	4761-02	breast	DCIS	G0	S.E. I. Pathology		+1	-1	+9
17	16	Block1	657-03	breast	connective	G0	Bay		+2	6	+7
18	17	Block1	1222-03	breast	IDC	G2	Bay		+1	5	-2
19	18	Block1	1324-03	breast	ILC	G1	Bay		+2	3	0
20	19	Block1	1643-04	breast	IDC	G2	Bay		+1	2	+1
21	20	Block1	1643-04	breast	IDC	G2	Bay		0	5	+9
22	21	Block1	1826-02	breast	DCIS	G0	S.E. I. Pathology		+1	4	+7
23	22	Block1	1877-02	breast	ILC	G2	Natl. Inst. Cancer		0	5	+2
24	23	Block1	988-03	breast	IDC	G2	Bay		+1	4	+1
25	24	Block1	1023-03	breast	IDC	G0	Bay		0	3	+2
26	25	Block2	210-05	breast	connective	G3	Bay		+1	4	0
27	26	Block2	1643-04	breast	ILC	G2	Bay		0	6	-2
28	27	Block2	1416-04	breast	IDC	G2	S.E. I. Pathology		0	7	+1
29	28	Block2	1610-04	breast	IDC	G3	S.E. I. Pathology		0	6	+7
30	29	Block2	128-05	breast	IDC	G3	S.E. I. Pathology		0	7	+1
31	30	Block2	774-02	breast	DCIS	G2	S.E. I. Pathology		0	3	0
32	31	Block2	1315-02	breast	connective	G2	S.E. I. Pathology		-2	4	-2

Allred Value **= Intensity** Score Value **+ Rate** Score Value

For example, in the case of 8: 4 (Rate) + 2 (Intensity) = 6 (Allred)



6.1.4 Exporting Clusters into XLS

It makes possible to save the Clusters into XLS by the software.

This export type is only valid if the slide was scored with a Combined Scoring Scheme and you have also set Thresholds in the Scheme.

	A	B	C	D	E	F	G	H	I	J	K
1	Unique TMA Sample ID	TMA Block ID	Donor Block ID	Organ	Diagnosis	Grade	Sender in	Survival	B-Catenin	Cox-2_Cluster	EGFR-Ventana
2	1	Block1	1722-02	liver	cirrhosis	G0	Szombathely			+2	
3	2	Block1	546-04	breast	IDC	G2	Bay			+3	
4	3	Block1	546-04	breast	IDC	G2	Bay			+2	
5	4	Block1	210-04	breast	Mucinosus	G2	Bay			+1	
6	5	Block1	210-05	breast	IDC	G3	Bay			+2	
7	6	Block1	1643-04	breast	IDC	G3	Bay			+1	
8	7	Block1	1416-04	breast	ILC	G2	Bay			+1	
9	8	Block1	1610-04	breast	IDC, come	G2	Bay			+2	
10	9	Block1	128-05	breast	ADC meta	poor	Bay			+2	
11	10	Block1	774-02	breast	connective	G0	S.E. I. Pathology			+1	
12	11	Block1	659-02	breast	ILC	G3	S.E. I. Pathology			+3	
13	12	Block1	322-02	breast	IDC	G2	S.E. I. Pathology				
14	13	Block1	1315-02	breast	IDC	G2	S.E. I. Pathology				
15	14	Block1	1199-02	breast	IDC	G3	S.E. I. Pathology				
16	15	Block1	4761-02	breast	DCIS	G0	S.E. I. Pathology				
17	16	Block1	657-03	breast	connective	G0	Bay			+3	
18	17	Block1	1222-03	breast	IDC	G2	Bay			+2	
19	18	Block1	1324-03	breast	ILC	G1	Bay			+1	
20	19	Block1	1643-04	breast	IDC	G2	Bay			+1	
21	20	Block1	1643-04	breast	IDC	G2	Bay			+3	
22	21	Block1	1826-02	breast	DCIS	G0	S.E. I. Pathology			+2	
23	22	Block1	1877-02	breast	ILC	G2	Natl. Inst. Cancer			+2	
24	23	Block1	988-03	breast	IDC	G2	Bay			+1	
25	24	Block1	1023-03	breast	IDC	G0	Bay			+1	
26	25	Block2	210-05	breast	connective	G3	Bay			+2	
27	26	Block2	1643-04	breast	ILC	G2	Bay			+3	
28	27	Block2	1416-04	breast	IDC	G2	S.E. I. Pathology			+3	
29	28	Block2	1610-04	breast	IDC	G3	S.E. I. Pathology			+3	
30	29	Block2	128-05	breast	IDC	G3	S.E. I. Pathology			+3	
31	30	Block2	774-02	breast	DCIS	G2	S.E. I. Pathology			+1	
32	31	Block2	1315-02	breast	connective	G2	S.E. I. Pathology			+2	

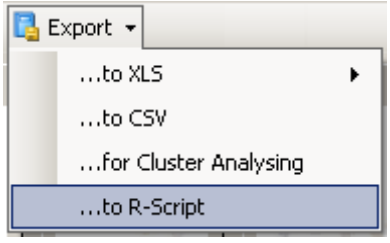
6.1.5 Exporting Score Values for Cluster Analyzing

This type of Exporting generates a special .txt file for the TreeView software (see below). It automatically generates a "GWEIGHT" column, and an "EWEIGHT" row. The columns selected by the user are displayed in one column, each columns separated with a "|" symbol. The points related to different stainings are displayed in separate columns. The file can only be imported in this format to the TreeView software, for further processing.

	A	B	C	D	E	F	G
1	UID	NAME	GWEIGHT	B-Catenin	Cox-2	EGFR-Ventana	p53-SP5
2	EWEIGHT			1	1	1	1
3	1	1| Block1 | 1722-02 | liver | cirrhosis | G0 | Szombathely | | |	1	-2	7	2	3
4	2	2| Block1 | 546-04 | breast | IDC | G2 | Bay | | |	1	1	11	9	6
5	3	3| Block1 | 546-04 | breast | IDC | G2 | Bay | | |	1	1	7	9	9
6	4	4| Block1 | 210-04 | breast | Mucinosus carcinoma | G2 | Bay | | |	1	2	3	7	10
7	5	5| Block1 | 210-05 | breast | IDC | G3 | Bay | | |	1	1	7	2	13
8	6	6| Block1 | 1643-04 | breast | IDC | G3 | Bay | | |	1	2	3	1	7
9	7	7| Block1 | 1416-04 | breast | ILC | G2 | Bay | | |	1	1	3	2	12
10	8	8| Block1 | 1610-04 | breast | IDC, comedo | G2 | Bay | | |	1	0	6	1	16
11	9	9| Block1 | 128-05 | breast | ADC metastasis | poor | Bay | | |	1	1	9	0	10
12	10	10| Block1 | 774-02 | breast | connective tissue | G0 | S.E. I. Pathology | | |	1	0	5	-2	10
13	11	11| Block1 | 659-02 | breast | ILC | G3 | S.E. I. Pathology | | |	1	-2	10	7	5
14	12	12| Block1 | 322-02 | breast | IDC | G2 | S.E. I. Pathology | | |	1	0		2	1
15	13	13| Block1 | 1315-02 | breast | IDC | G2 | S.E. I. Pathology | | |	1	-2	0	0	3
16	14	14| Block1 | 1199-02 | breast | IDC | G3 | S.E. I. Pathology | | |	1	0		1	6
17	15	15| Block1 | 4761-02 | breast | DCIS | G0 | S.E. I. Pathology | | |	1	1	-1	9	8
18	16	16| Block1 | 657-03 | breast | connective tissue | G0 | Bay | | |	1	2	8	7	
19	17	17| Block1 | 1222-03 | breast | IDC | G2 | Bay | | |	1	1	4	-2	10
20	18	18| Block1 | 1324-03 | breast | ILC | G1 | Bay | | |	1	2	2	0	16
21	19	19| Block1 | 1643-04 | breast | IDC | G2 | Bay | | |	1	1	1	1	11
22	20	20| Block1 | 1643-04 | breast | IDC | G2 | Bay | | |	1	0	10	9	2
23	21	21| Block1 | 1826-02 | breast | DCIS | G0 | S.E. I. Pathology | | |	1	1	6	7	9
24	22	22| Block1 | 1877-02 | breast | ILC | G2 | Natl. Inst. Cancer | | |	1	0	7	2	14
25	23	23| Block1 | 988-03 | breast | IDC | G2 | Bay | | |	1	1	3	1	4
26	24	24| Block1 | 1023-03 | breast | IDC | G0 | Bay | | |	1	0	2	2	8
27	25	25| Block2 | 210-05 | breast | connective tissue | G3 | Bay | | |	1	1	6	0	11
28	26	26| Block2 | 1643-04 | breast | ILC | G2 | Bay | | |	1	0	11	-2	10
29	27	27| Block2 | 1416-04 | breast | IDC | G2 | S.E. I. Pathology | | |	1	0	12	1	6

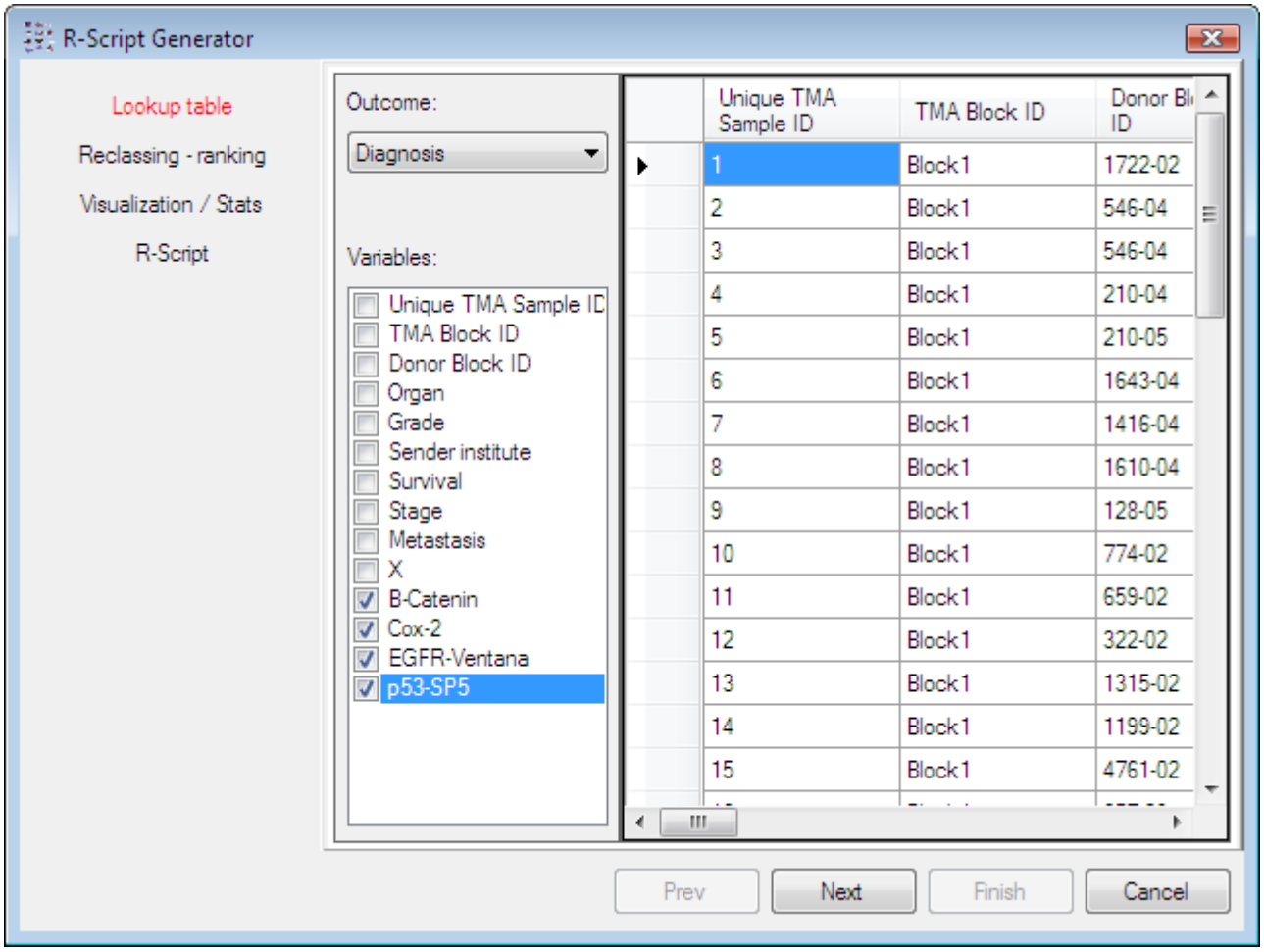
6.1.6 Exporting Score Values to R Software

R is a statistics-producing software that is able to predict an estimated life expectancy on the base of the exported to it Score Values, using its enormous fact-database.



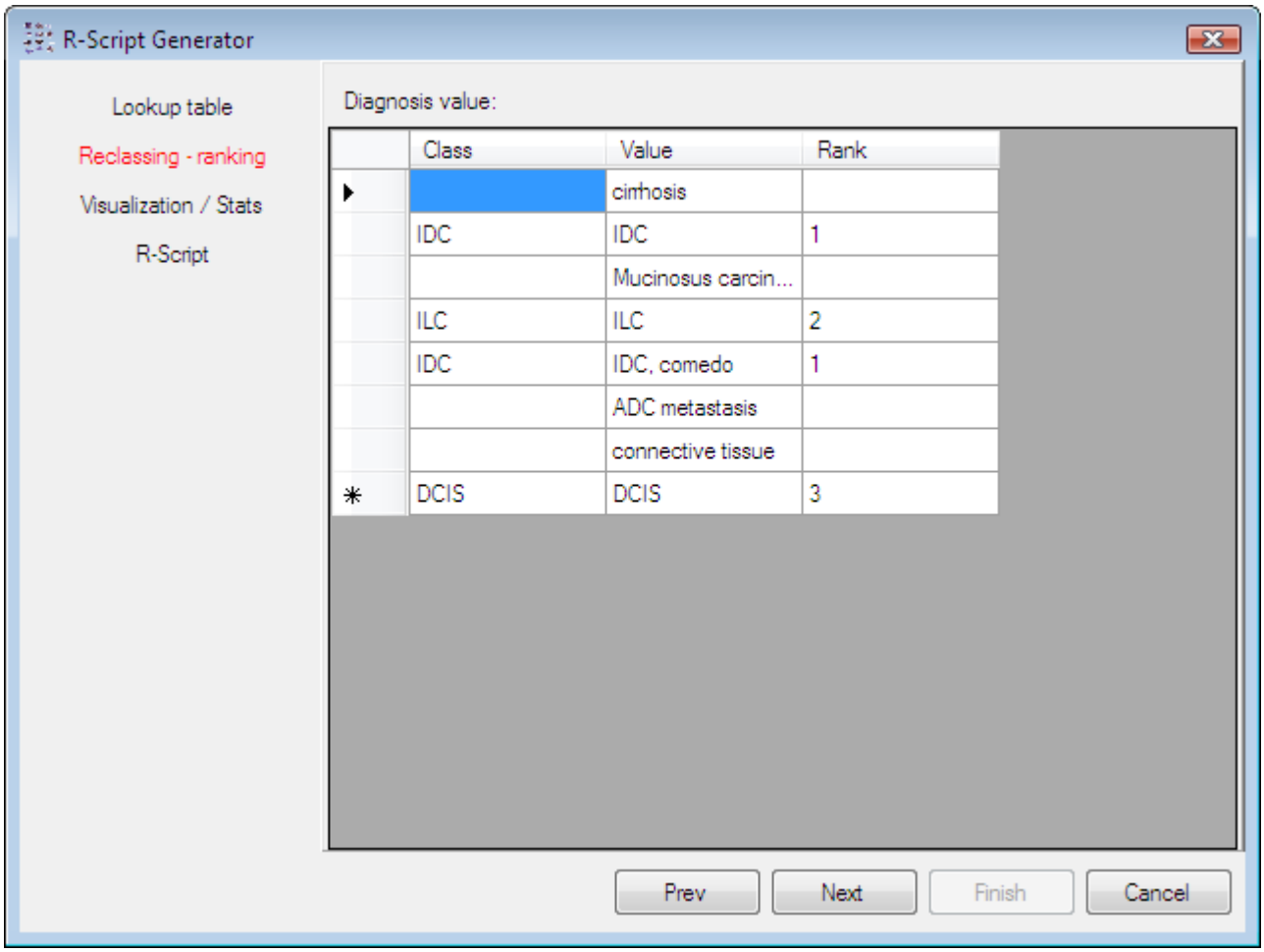
Lookup table

Select the points, the criteria of the statistical analysis from the panel, then mark the needed variables for the statistical calculations. When finished, click **Next**.



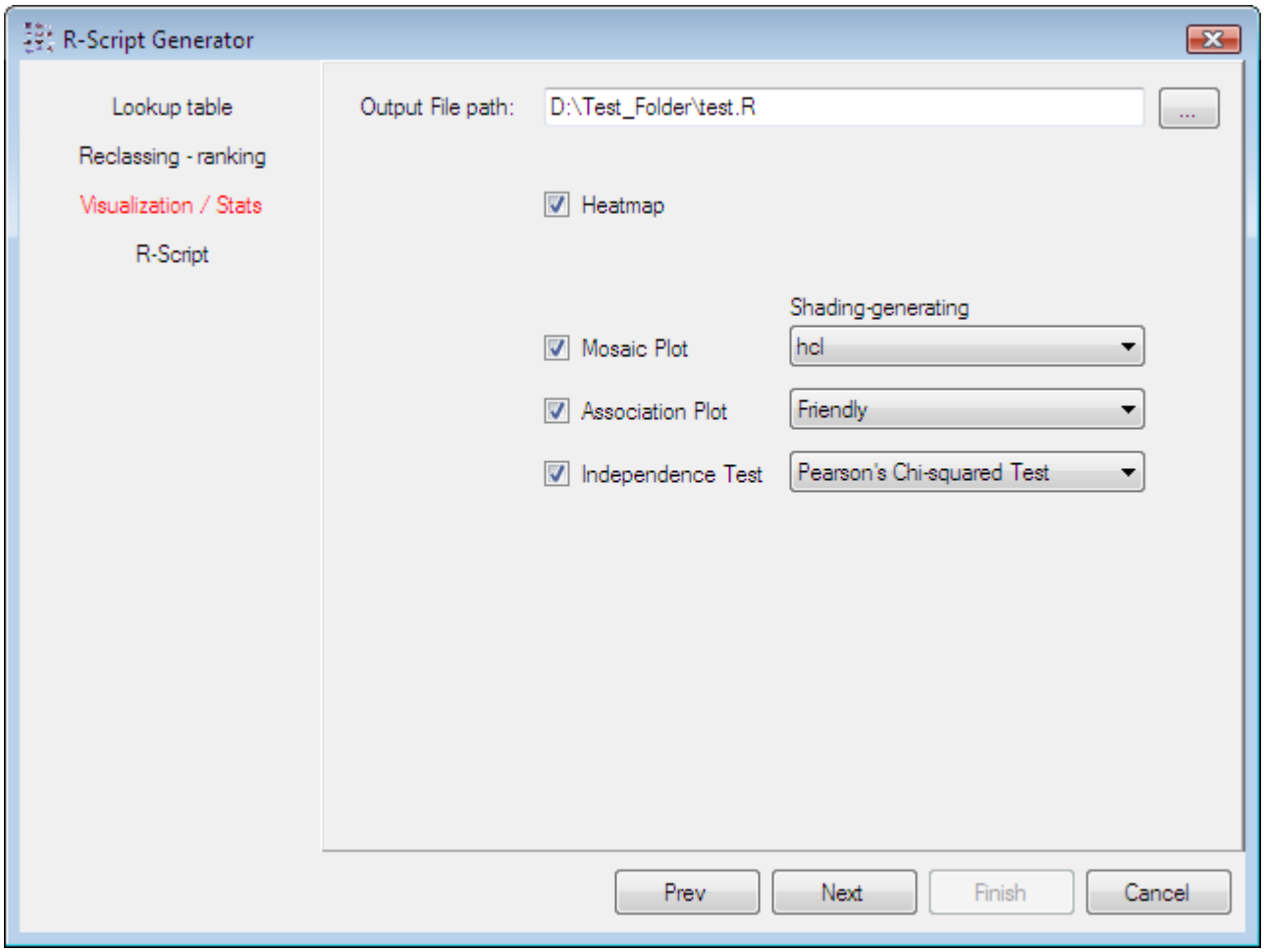
Reclassing - ranking

The software displays an arrangement of the chosen criteria that you can re-arrange. Make an order of importance for these criteria. To enter new values or to modify them, double-click on the field. When finished, click **Next**.



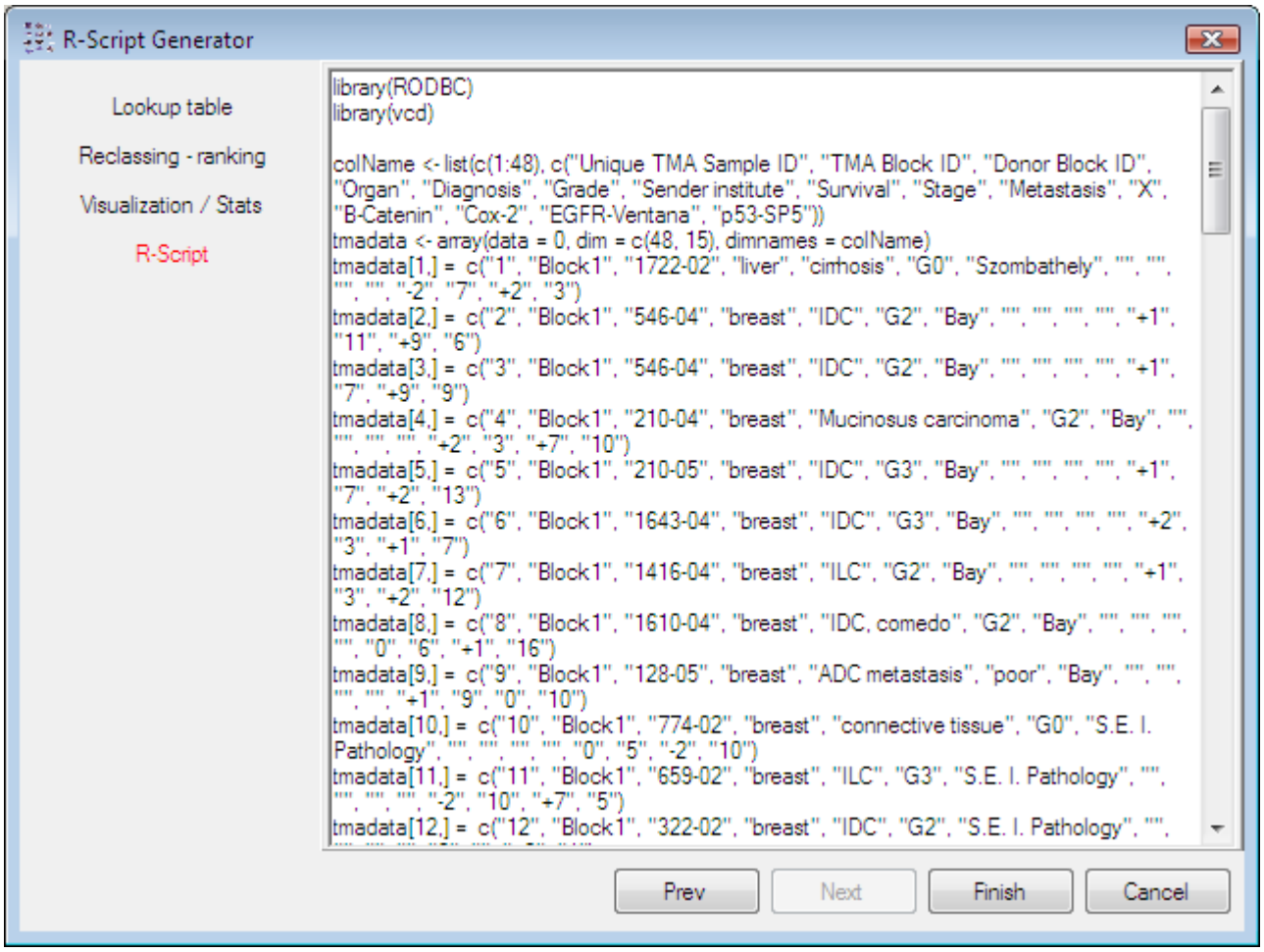
Visualization / Stats

Define the folder and the file to where you want to save the R-script. If you want to visualize the statistical results, mark (tick) the options Heatmap, Mosaic Plot, Association Plot. When finished, click **Next**.



R-Script

The generated R-script is displayed. To save it into the formerly defined folder and file click Finish.


R-Script Generator
Lookup table
Reclassing - ranking
Visualization / Stats
R-Script
library(RODBC)
library(vcd)
colName <- list(c(1:48), c("Unique TMA Sample ID", "TMA Block ID", "Donor Block ID", "Organ", "Diagnosis", "Grade", "Sender institute", "Survival", "Stage", "Metastasis", "X", "B-Catenin", "Cox-2", "EGFR-Ventana", "p53-SP5"))
tmadata <- array(data = 0, dim = c(48, 15), dimnames = colName)
tmadata[1,] = c("1", "Block1", "1722-02", "liver", "cirrhosis", "G0", "Szombathely", "", "", "", "", "-2", "7", "+2", "3")
tmadata[2,] = c("2", "Block1", "546-04", "breast", "IDC", "G2", "Bay", "", "", "", "", "+1", "11", "+9", "6")
tmadata[3,] = c("3", "Block1", "546-04", "breast", "IDC", "G2", "Bay", "", "", "", "", "+1", "7", "+9", "9")
tmadata[4,] = c("4", "Block1", "210-04", "breast", "Mucinosus carcinoma", "G2", "Bay", "", "", "", "", "+2", "3", "+7", "10")
tmadata[5,] = c("5", "Block1", "210-05", "breast", "IDC", "G3", "Bay", "", "", "", "", "+1", "7", "+2", "13")
tmadata[6,] = c("6", "Block1", "1643-04", "breast", "IDC", "G3", "Bay", "", "", "", "", "+2", "3", "+1", "7")
tmadata[7,] = c("7", "Block1", "1416-04", "breast", "ILC", "G2", "Bay", "", "", "", "", "+1", "3", "+2", "12")
tmadata[8,] = c("8", "Block1", "1610-04", "breast", "IDC, comedo", "G2", "Bay", "", "", "", "", "0", "6", "+1", "16")
tmadata[9,] = c("9", "Block1", "128-05", "breast", "ADC metastasis", "poor", "Bay", "", "", "", "", "+1", "9", "0", "10")
tmadata[10,] = c("10", "Block1", "774-02", "breast", "connective tissue", "G0", "S.E. I. Pathology", "", "", "", "", "0", "5", "-2", "10")
tmadata[11,] = c("11", "Block1", "659-02", "breast", "ILC", "G3", "S.E. I. Pathology", "", "", "", "", "-2", "10", "+7", "5")
tmadata[12,] = c("12", "Block1", "322-02", "breast", "IDC", "G2", "S.E. I. Pathology", "",
Prev
Next
Finish
Cancel

(For more information, visit the homepage http://www.r-project.org/)

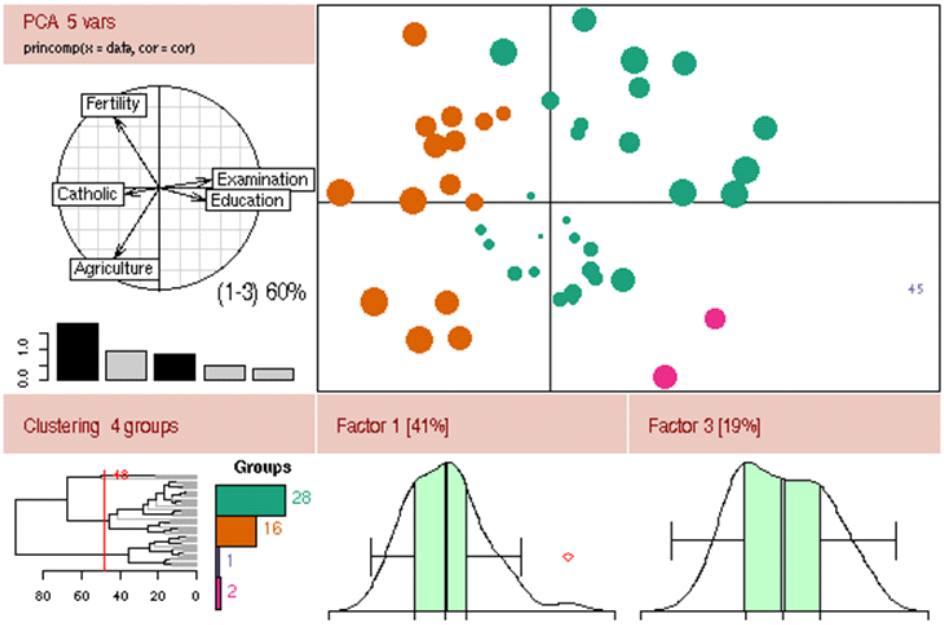
The Project for Statistical Computing

Getting Started:

- R is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS. To download R, select

your preferred CRAN mirror.

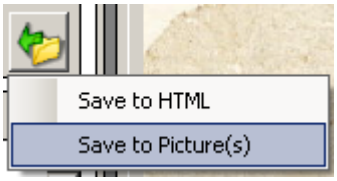
- If you have questions about R like how to download and install the software, or what the license terms are, read our answers to frequently asked questions before you send an email.



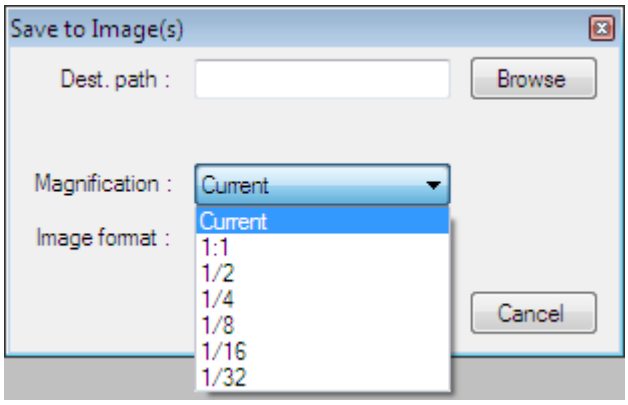
This server is hosted by the Department of Statistics and Mathematics of the WU Wien.)

6.2 Exporting Images

Using the Export Images button in the Gallery allows for saving TMA spot image collections either in JPEG, BMP, TIFF or PNG formats, or as an HTML page (in JPEG).



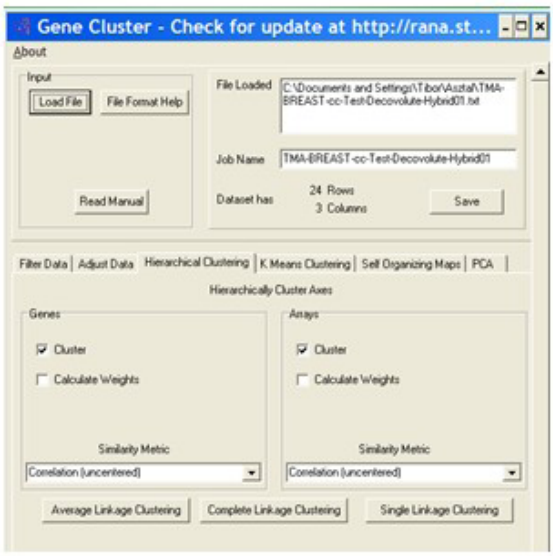
Images can be saved at arbitrary magnifications and new directories can be defined.

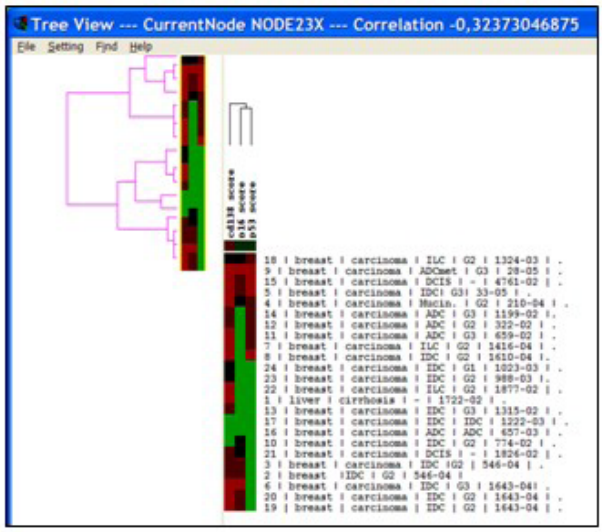


7 Analyzing the Data Exported from TMA Module (Cluster Analysis)

For Cluster Analysis --- without using the Deconvoluter program --- the 4-scale system above (-2 = negative, 0 = equivocal, 1 = weak-moderate positive, 2 = strongly positive) has to be used. At Saving Data to Excel, the program will convert them into a format, which can be loaded and analyzed in the Cluster program (for example version 2.11, see below). The modifier columns intended to appear in TreeView (for example version 1.60, see below) are also merged into one column and all other columns except those with the scores, are deleted.

The modified Lookup table above is saved as a text file (data divided by tabs), which can be Loaded into the Gene Cluster program. Average Linkage Clustering (Correlation uncentered) under the option of Hierarchical Clustering will result in relation analyses, which can be imported and viewed in heat-map and dendrogram representations using the TreeView program.





Recommended software tools used for Hierarchical Clustering Analysis of TMA data may be downloaded freely from the following web pages:

TMA Deconvoluter (1.11)

http://genome-www.stanford.edu/TMA/download.shtml, by Liu CL, Prapong W, Natkunam Y, Alizadeh A, Montgomery K, Gilks CB, van de Rijn M (2002) TMA Deconvoluter (1.11) software by Software Tools for High-Throughput Analysis and Archiving of Immunohistochemistry Staining Data Obtained with Tissue Microarrays American Journal of Pathology 61:1557-1565.)

The conversion was done with the TMA module for MIRAX Viewer 1.12.

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